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(71) Applicants (for all designated States except US): FAS-
GEN, LLC. [US/US]; Baview Medical Campus, 5210
Eastern Avenue, Baltimore, MD 21224 (US). THE
JOHNS HOPKINS UNIVERSITY [US/US]; 4910
Eastern Avenue, Baltimore, MD 21224 (US).

(72) Inventors; and

(75) Inventors/Applicants (for US only): KUHADJA,
Francis, P. [US/US]; Johns Hopkins Medical Center,
4940 Eastern Avenue, Baltimore, MD 21224 (US).
MEDGHALCHI, Susan, M. [US/US]; Fasgen, Inc.,
Alpha Center, Johns Hopkins Bayview Research, 5210
Eastern Avenue, Baltimore, MD 21224 (US). THUPARI,
Jagan, N. [US/US]; Department of Pathology, Johns
Hopkins Bayview Research Campus, 5210 Eastern Av-
enue, Baltimore, MD 21224 (US). TOWNSEND, Craig,
A. [US/US]; Department of Chemistry, Johns Hopkins

University, 3400 N. Charles Street, Baltimore, MD 21218
(US). McFADDEN, Jill, M. [US/US]; The Johns Hopkins
University, Department of Chemistry, 3400 Charles Street,
Baltimore, MD 21218 (US).

(74) Agents: STIEFEL, Maurice, B. et al.; Bryan Cave LLP,
1290 Avenue of the Americas, New York, NY 10167 (US).

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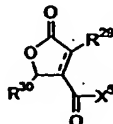
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(54) Title: NOVEL COMPOUNDS, PHARMACEUTICAL COMPOSITIONS CONTAINING SAME, AND METHODS OF USE
FOR SAME



(IX)

(57) Abstract: Pharmaceutical composition comprising a pharmaceutical diluent and a compound of formula IX: R²⁹ = H, or C₁-C₂₀ alkyl, cycloalkyl, alkenyl, aryl, arylalkyl, or alkylaryl, =CHR³¹, -C(O)OR³¹, -C(O)R³¹, -CH₂C(O)OR³¹, CH₂C(O)NHR³¹, where R³¹ is H or C₁-C₁₀ alkyl, cycloalkyl, or alkenyl; R³⁰ = C₁-C₂₀ alkyl, cycloalkyl, alkenyl, aryl, arylalkyl, or alkylaryl; X⁵ = -OR³², or NHR³², where R³² is H, C₁-C₂₀ alkyl, cycloalkyl, alkenyl, aryl, arylalkyl, or alkylaryl, the R³² group optionally containing a carbonyl group, a carboxyl group, a carboxyamide group, an alcohol group, or an ether group, the R³² group further optionally containing one or more halogen atoms; with the proviso that when R²⁹ is =CH₂, then X⁵ is not OH. Also disclosed are compounds within the scope of the formula IX, as well as uses of the pharmaceutical compositions for weight loss, anti-microbial and anti-cancer applications, inhibition of fatty acid synthase and neuropeptide-Y, and the stimulation of the activity of carnitine palmitoyl transferase-1.

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NOVEL COMPOUNDS, PHARMACEUTICAL COMPOSITIONS CONTAINING SAME, AND METHODS OF USE FOR SAME

BACKGROUND OF THE INVENTION

Fatty acid synthase

5 Fatty acids have three primary roles in the physiology of cells. First, they are the building blocks of biological membranes. Second, fatty acid derivatives serve as hormones and intracellular messengers. Third, and of particular importance to the present invention, fatty acids are fuel molecules that can be stored in adipose tissue as triacylglycerols, which are also known
10 as neutral fats.

There are four primary enzymes involved in the fatty acid synthetic pathway, fatty acid synthase (FAS), acetyl CoA carboxylase (ACC), malic enzyme, and citric lyase. The principal enzyme, FAS, catalyzes the NADPH-dependent condensation of the precursors malonyl-CoA and acetyl-CoA to produce fatty acids. NADPH is a reducing agent that generally
15 serves as the essential electron donor at two points in the reaction cycle of FAS. The other three enzymes (i.e., ACC, malic enzyme, and citric lyase) produce the necessary precursors. Other enzymes, for example the enzymes that produce NADPH, are also involved in fatty acid synthesis.

FAS has an Enzyme Commission (E.C.) No. 2.3.1.85 and is also known as fatty
20 acid synthetase, fatty acid ligase, as well as its systematic name acyl-CoA:malonyl-CoA C-acyltransferase (decarboxylating, oxoacyl- and enoyl-reducing and thioester-hydrolysing). There are seven distinct enzymes – or catalytic domains - involved in the FAS catalyzed synthesis of fatty acids: acetyl transacylase, malonyl transacylase, beta-ketoacyl synthetase (condensing enzyme), beta-ketoacyl reductase, beta-hydroxyacyl dehydrase, enoyl reductase, and thioesterase.

(Wakil, S. J., Biochemistry, 28: 4523-4530, 1989). All seven of these enzymes together form FAS.

Although the FAS catalyzed synthesis of fatty acids is similar in lower organisms, such as, for example, bacteria, and in higher organisms, such as, for example, mycobacteria, yeast and humans, there are some important differences. In bacteria, the seven enzymatic reactions are carried out by seven separate polypeptides that are non-associated. This is classified as Type II FAS. In contrast, the enzymatic reactions in mycobacteria, yeast and humans are carried out by multifunctional polypeptides. For example, yeast have a complex composed of two separate polypeptides whereas in mycobacterium and humans, all seven reactions are carried out by a single polypeptide. These are classified as Type I FAS.

FAS inhibitors

Various compounds have been shown to inhibit fatty acid synthase (FAS). FAS inhibitors can be identified by the ability of a compound to inhibit the enzymatic activity of purified FAS. FAS activity can be assayed by measuring the incorporation of radiolabeled precursor (i.e., acetyl-CoA or malonyl-CoA) into fatty acids or by spectrophotometrically measuring the oxidation of NADPH. (Dils, et al., Methods Enzymol., 35:74-83).

Table-1, set forth below, lists several FAS inhibitors.

Table 1**Representative Inhibitors Of The Enzymes Of The Fatty Acid Synthesis Pathway**

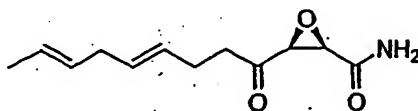
<u>Inhibitors of Fatty Acid Synthase</u> 1,3-dibromopropanone Ellman's reagent (5,5'-dithiobis(2-nitrobenzoic acid), DTNB) 4-(4'-chlorobenzoyloxy) benzyl nicotinate (KCD-232) 4-(4'-chlorobenzoyloxy) benzoic acid (MII) 2(5(4-chlorophenyl)pentyl)oxirane-2-carboxylate (POCA) and its CoA derivative ethoxyformic anhydride	cerulenin phenyocerulenin melarsoprol iodoacetate phenylarsineoxide pentostam melittin thiolactomycin
<u>Inhibitors for citrate lyase</u> (-) hydroxycitrate (R,S)-S-(3,4-dicarboxy-3-hydroxy-3-methyl-butyl)-CoA S-carboxymethyl-CoA	<u>Inhibitors for malic enzyme</u> periodate-oxidized 3-aminopyridine adenine dinucleotide phosphate 5,5'-dithiobis(2-nitrobenzoic acid) p-hydroxymercuribenzoate N-ethylmaleimide oxalyl thiol esters such as S-oxalylglutathione gossypol phenylglyoxal 2,3-butanedione bromopyruvate pregnenolone
<u>Inhibitors for acetyl CoA carboxylase</u> sethoxydim haloxyfop and its CoA ester diclofop and its CoA ester clethodim alloxydim trifop clofibric acid 2,4-D mecoprop dalapon 2-alkyl glutarate 2-tetradecanylethylglutamate (TDG) 2-octylethylglutamate N6,02-dibutyladenosine cyclic 3',5'-monophosphate N2,02-dibutyladenosine cyclic 3',5'-monophosphate CoA derivative of 5-(tetradecyloxy)-2-furoic acid (TOFA) 2,3,7,8-tetrachlorodibenzo-p-dioxin	9-decenyl-1-pentenedioic acid decanyl-2-pentenedioic acid decanyl-1-pentenedioic acid (S)-ibuprofenyl-CoA (R)-ibuprofenyl-CoA fluazifop and its CoA ester clofop 5-(tetradecyloxy)-2-furoic acid beta, beta'-tetramethylhexadecanedioic acid tralkoxydim free or monothioester of beta, beta prime-methyl-substituted hexadecanedioic acid (MEDICA 16) alpha-cyano-4-hydroxycinnamate S-(4-bromo-2,3-dioxobutyl)-CoA p-hydroxymercuribenzoate (PHMB) N6,02-dibutyladenosine cyclic 3',5'-monophosphate

Of the four enzymes in the fatty acid synthetic pathway, FAS is the preferred target for inhibition because it acts only within the pathway to fatty acids, while the other three enzymes are implicated in other cellular functions. Therefore, inhibition of one of the other three enzymes is more likely to affect normal cells. Of the seven enzymatic steps carried out by FAS, the step catalyzed by the condensing enzyme (*i.e.*, beta-ketoacyl synthetase) and the enoyl reductase have been the most common candidates for inhibitors that reduce or stop fatty acid synthesis. The condensing enzyme of the FAS complex is well characterized in terms of structure and function. The active site of the condensing enzyme contains a critical cysteine thiol, which is the target of antilipidemic reagents, such as, for example, the inhibitor cerulenin.

Preferred inhibitors of the condensing enzyme include a wide range of chemical compounds, including alkylating agents, oxidants, and reagents capable of undergoing disulphide exchange. The binding pocket of the enzyme prefers long chain, *E, E*, dienes.

In principal, a reagent containing the sidechain diene and a group which exhibits reactivity with thiolate anions could be a good inhibitor of the condensing enzyme. Cerulenin

[(2*S*, 3*R*)-2,3-epoxy-4-oxo-7,10 dodecadienoyl amide] is an example:



Cerulenin covalently binds to the critical cysteine thiol group in the active site of the condensing enzyme of fatty acid synthase, inactivating this key enzymatic step (Funabashi, et al., J.

Biochem., 105:751-755, 1989). While cerulenin has been noted to possess other activities, these

either occur in microorganisms which may not be relevant models of human cells (*e.g.*, inhibition of cholesterol synthesis in fungi, Omura (1976), Bacteriol. Rev., 40:681-697; or diminished RNA synthesis in viruses, Perez, et al. (1991), FEBS, 280: 129-133), occur at a substantially higher

drug concentrations (inhibition of viral HIV protease at 5 mg/ml, Moelling, et al. (1990), FEBS, 261:373-377) or may be the direct result of the inhibition of endogenous fatty acid synthesis (inhibition of antigen processing in B lymphocytes and macrophages, Faló, et al. (1987), J. Immunol., 139:3918-3923). Some data suggest that cerulenin does not specifically inhibit myristoylation of proteins (Simon, et al., J. Biol. Chem., 267:3922-3931, 1992).

Several more FAS inhibitors are disclosed in U.S. Patent Application No. 08/096,908 and its CIP filed Jan. 24, 1994, the disclosures of which are hereby incorporated by reference. Included are inhibitors of fatty acid synthase, citrate lyase, CoA carboxylase, and malic enzyme.

Tomoda and colleagues (Tomoda et al., Biochim. Biophys. Act 921:595-598 1987; Omura et al., J. Antibiotics 39:1211-1218 1986) describe Triacsin C (sometimes termed WS-1228A), a naturally occurring acyl-CoA synthetase inhibitor, which is a product of *Streptomyces* sp. SK-1894. The chemical structure of Triacsin C is 1-hydroxy-3-(*E, E, E*-2',4',7'-undecatrienylidene) triazene. Triacsin C causes 50% inhibition of rat liver acyl-CoA synthetase at 8.7 μ M; a related compound, Triacsin A, inhibits acyl CoA-synthetase by a mechanism which is competitive with long-chain fatty acids. Inhibition of acyl-CoA synthetase is toxic to animal cells. Tomoda et al. (Tomoda et al., J. Biol. Chem. 266:4214-4219, 1991) teaches that Triacsin C causes growth inhibition in Raji cells at 1.0 μ M, and have also been shown to inhibit growth of Vero and Hela cells. Tomoda et al. further teaches that acyl-CoA synthetase is essential in animal cells and that inhibition of the enzyme has lethal effects.

A family of compounds (gamma-substituted-alpha-methylene-beta-carboxy-gamma-butyrolactones) has been shown in U.S. Patent No. 5,981,575 (the disclosure of which is hereby incorporated by reference) to inhibit fatty acid synthesis, inhibit growth of tumor cells,

and induce weight loss. The compounds disclosed in the '575 Patent have several advantages over the natural product cerulenin for therapeutic applications: [1] they do not contain the highly reactive epoxide group of cerulenin, [2] they are stable and soluble in aqueous solution, [3] they can be produced by a two-step synthetic reaction and thus easily produced in large quantities, and [4] they are easily tritiated to high specific activity for biochemical and pharmacological analyses. The synthesis of this family of compounds, which are fatty acid synthase inhibitors, is described in the '575 Patent, as is their use as a means to treat tumor cells expressing FAS, and their use as a means to reduce body weight. The '575 Patent also discloses the use of any fatty acid synthase inhibitors to systematically reduce adipocyte mass (adipocyte cell number or size) as a means to reduce body weight.

The primary sites for fatty acid synthesis in mice and humans are the liver (*see* Roncari, *Can. J. Biochem.*, 52:221-230, 1974; Triscari et al., 1985, *Metabolism*, 34:580-7; Barakat et al., 1991, *Metabolism*, 40:280-5), lactating mammary glands (*see* Thompson, et al., *Pediatr. Res.*, 19:139-143, 1985) and adipose tissue (Goldrick et al., 1974, *Clin. Sci. Mol. Med.*, 46:469-79).

Inhibitors of fatty acid synthesis as antimicrobial agents

Cerulenin was originally isolated as a potential antifungal antibiotic from the culture broth of *Cephalosporium caerulens*. Structurally cerulenin has been characterized as (2*R*,3*S*)-epoxy-4-oxo-7,10-trans,trans-dodecanoic acid amide. Its mechanism of action has been shown to be inhibition, through irreversible binding, of beta-ketoacyl-ACP synthase, the condensing enzyme required for the biosynthesis of fatty acids. Cerulenin has been categorized as an antifungal, primarily against *Candida* and *Saccharomyces sp.* In addition, some in vitro

activity has been shown against some bacteria, actinomycetes, and mycobacteria, although no activity was found against *Mycobacterium tuberculosis*. The activity of fatty acid synthesis inhibitors and cerulenin in particular has not been evaluated against protozoa such as *Toxoplasma gondii* or other infectious eucaryotic pathogens such as *Pneumocystis carinii*,

5 *Giardia lamblia*, *Plasmodium sp.*, *Trichomonas vaginalis*, *Cryptosporidium*, *Trypanosoma*, *Leishmania*, and *Schistosoma*.

Infectious diseases which are particularly susceptible to treatment are diseases which cause lesions in externally accessible surfaces of the infected animal. Externally accessible surfaces include all surfaces that may be reached by non-invasive means (without cutting or
10 puncturing the skin), including the skin surface itself, mucus membranes, such as those covering nasal, oral, gastrointestinal, or urogenital surfaces, and pulmonary surfaces, such as the alveolar sacs. Susceptible diseases include: (1) cutaneous mycoses or tineas, especially if caused by *Microsporum*, *Trichophyton*, *Epidermophyton*, or *Mucocutaneous candidiasis*; (2) mucotic keratitis, especially if caused by *Aspergillus*, *Fusarium* or *Candida*; (3) amoebic keratitis,
15 especially if caused by *Acanthamoeba*; (4) gastrointestinal disease, especially if caused by *Giardia lamblia*, *Entamoeba*, *Cryptosporidium*, *Microsporidium*, or *Candida* (most commonly in immunocompromised animals); (5) urogenital infection, especially if caused by *Candida albicans* or *Trichomonas vaginalis*; and (6) pulmonary disease, especially if caused by *Mycobacterium tuberculosis*, *Aspergillus*, or *Pneumocystis carinii*. Infectious organisms that are
20 susceptible to treatment with fatty acid synthesis inhibitors include *Mycobacterium tuberculosis*, especially multiply-drug resistant strains, and protozoa such as *Toxoplasma*.

Any compound that inhibits fatty acid synthesis may be used to inhibit microbial cell growth. However, compounds administered to a patient must not be equally toxic to both

patient and the target microbial cells. Accordingly, it is beneficial to select inhibitors that only, or predominantly, affect target microbial cells.

Eukaryotic microbial cells which are dependent on their own endogenously synthesized fatty acid will express Type I FAS. This is shown both by the fact that FAS inhibitors are growth inhibitory and by the fact that exogenously added fatty acids can protect normal patient cells but not these microbial cells from FAS inhibitors. Therefore, agents which prevent synthesis of fatty acids by the cell may be used to treat infections. In eukaryotes, fatty acids are synthesized by Type I FAS using the substrates acetyl CoA, malonyl CoA and NADPH. Thus, other enzymes which can feed substrates into this pathway may also effect the rate of fatty acid synthesis and thus be important in microbes that depend on endogenously synthesized fatty acid. Inhibition of the expression or activity of any of these enzymes will effect growth of the microbial cells that are dependent upon endogenously synthesized fatty acid.

The product of Type I FAS differs in various organisms. For example, in the fungus *S. cerevisiae* the products are predominately palmitate and stearate esterified to coenzyme-A. In *Mycobacterium smegmatis*, the products are saturated fatty acid CoA esters ranging in length from 16 to 24 carbons. These lipids are often further processed to fulfill the cells need for various lipid components.

Inhibition of key steps in down-stream processing or utilization of fatty acids may be expected to inhibit cell function, whether the cell depends on endogenous fatty acid or utilizes fatty acid supplied from outside the cell, and so inhibitors of these down-stream steps may not be sufficiently selective for microbial cells that depend on endogenous fatty acid. However, it has been discovered that administration of Type I fatty acid synthesis inhibitor to such microbes makes them more sensitive to inhibition by inhibitors of down-stream fatty acid processing.

and/or utilization. Because of this synergy, administration of a fatty acid synthesis inhibitor in combination with one or more inhibitors of down-stream steps in lipid biosynthesis and/or utilization will selectively affect microbial cells that depend on endogenously synthesized fatty acid. Preferred combinations include an inhibitor of FAS and acetyl CoA carboxylase, or FAS
5 and an inhibitor of MAS.

When it has been determined that a mammal is infected with cells of an organism which expresses Type I FAS, or if FAS has been found in a biological fluid from a patient, the mammal or patient may be treated by administering a fatty acid synthesis inhibitor (Pat No. 5,614,551).

10 The inhibition of neuropeptide-Y to depress appetite and stimulate weight loss is described in International Patent Application No. PCT/US01/05316 the disclosure of which is hereby incorporated by reference. That application, however, does not describe or disclose any of the compounds disclosed in the present application

The stimulation of carnitine palmitoyl transferase-1 (CPT-1) to stimulate weight
15 loss is described in U.S. Patent Application Serial No. 60/354,480, the disclosure of which is hereby incorporated by reference. That application does not describe or disclose any of the compounds disclosed herein, either.

The use of FAS inhibitors to inhibit the growth of cancer cells is described in U.S. Patent No. 5,759,837, the disclosure of which is hereby incorporated by reference. That
20 application does not describe or disclose any of the compounds disclosed herein.

Summary of the Invention

New classes of compounds have been discovered which have a variety of therapeutically valuable properties, eg. FAS-inhibition, NPY-inhibition, CPT-1 stimulation, ability to induce weight loss, and anti-cancer and anti-microbial properties.

5 It is a further object of this invention to provide a method of inducing weight loss in animals and humans by administering a pharmaceutical composition comprising a pharmaceutical diluent and a compound of formula I, II, III, IV, V, VI, VII, VIII, or IX, which are described in detail below.

It is a further object of the invention to provide a method of stimulating the
10 activity of CPT-1 by administering to humans or animals a pharmaceutical composition comprising a pharmaceutical diluent and a compound of formula I, II, III, IV, V, VI, VII, VIII, or IX

It is a further object of the invention to provide a method of inhibiting the synthesis of neuropeptide Y in humans or animals by administering a pharmaceutical
15 composition comprising a pharmaceutical diluent and a compound of formula I, II, III, IV, V, VI, VII, VIII, or IX.

It is a further object of the invention to provide a method of inhibiting fatty acid synthase activity in humans or animals by administering a pharmaceutical composition comprising a pharmaceutical diluent and a compound of formula I, II, III, IV, V, VI, VII, VIII, or
20 IX.

It is a further object of this invention to provide a method of treating cancer in animals and humans by administering a pharmaceutical composition comprising a pharmaceutical diluent and a compound of formula I, II, III, IV, V, VI, VII, VIII, or IX.

It is still a further object of this invention to provide a method of preventing the growth of cancer cells in animals and humans by administering a pharmaceutical composition comprising a pharmaceutical diluent and a compound of formula I, II, III, IV, V, VI, VII, VIII, or IX.

5 It is a further object of this invention to provide a method of inhibiting growth of invasive microbial cells by administering a pharmaceutical composition comprising a pharmaceutical diluent and a compound of compound of formula I, II, III, IV, V, VI, VII, VIII, or IX.

10 **Brief Description of the Drawings**

FIG. 1 shows a synthetic scheme to make certain compounds according to the invention.

FIG. 2 shows a synthetic scheme to make certain compounds according to the invention.

15 FIG. 3 shows the results of *in vivo* testing of the anti-tumor properties of certain compounds according to the invention.

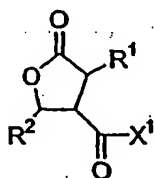
FIG. 4 shows the results of *in vivo* testing of the anti-tumor properties of a different compound according to the invention.

20 FIG. 5 shows the results of *in vivo* testing for weight loss of certain compounds according to the invention.

Detailed Description of the Invention

The compounds of the invention can be prepared by conventional means. The synthesis of a number of the compounds is described in the examples. The compounds may be useful for the treatment of obesity, cancer, or microbially-based infections.

5 One embodiment of the invention is compounds of formula I:



I

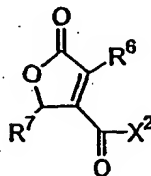
wherein

- $R^1 = \text{H, or } C_1\text{-}C_{20} \text{ alkyl, cycloalkyl, alkenyl, aryl, arylalkyl, or alkylaryl, } =\text{CHR}^3, -\text{C(O)OR}^3, -$
 10 $\text{C(O)R}^3, -\text{CH}_2\text{C(O)OR}^3, -\text{CH}_2\text{C(O)NHR}^3$, where R^3 is H or $C_1\text{-}C_{10}$ alkyl, cycloalkyl, or
 alkenyl;
 $R^2 = C_1\text{-}C_{20}$ alkyl, cycloalkyl, alkenyl, aryl, arylalkyl, or alkylaryl;
 $X^1 = \text{NHR}^4$, where R^4 is H, $C_1\text{-}C_{20}$ alkyl, cycloalkyl, alkenyl, aryl, arylalkyl, or alkylaryl, the R^4
 group optionally containing a carbonyl group, a carboxyl group, a carboxamide group,
 15 an alcohol group, or an ether group, the R^4 group further optionally containing one or
 more halogen atoms.

In a preferred embodiment, R^1 is $C_1\text{-}C_{10}$ alkyl, cycloalkyl, alkenyl, aryl, arylalkyl, or
 alkylaryl; or $=\text{CH}_2$. In a more preferred embodiment, R^1 is $-\text{CH}_3$ or $=\text{CH}_2$.

In another preferred embodiment, R^4 is $-\text{CH}_2\text{C(O)OR}^5$ or $-\text{CH}_2\text{C(O)NHR}^5$, where R^5 is
 20 $C_1\text{-}C_{10}$ alkyl, cycloalkyl, alkenyl, aryl, arylalkyl, or alkylaryl.

Another embodiment of the invention is compounds formula II



II

5 wherein

$R^6 = \text{H}$, or $\text{C}_1\text{-C}_{20}$ alkyl, cycloalkyl, alkenyl, aryl, arylalkyl, or alkylaryl, $-\text{C}(\text{O})\text{OR}^8$, $-\text{C}(\text{O})\text{R}^8$, $-\text{CH}_2\text{C}(\text{O})\text{OR}^8$, $-\text{CH}_2\text{C}(\text{O})\text{NHR}^8$, where R^8 is H or $\text{C}_1\text{-C}_{10}$ alkyl, cycloalkyl, or alkenyl;

$R^7 = \text{C}_1\text{-C}_{20}$ alkyl, cycloalkyl, alkenyl, aryl, arylalkyl, or alkylaryl;

$\text{X}^2 = \text{NHR}^9$, where R^9 is H , $\text{C}_1\text{-C}_{20}$ alkyl, cycloalkyl, alkenyl, aryl, arylalkyl, or alkylaryl, the R^9

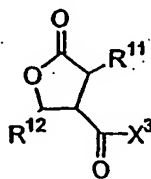
10 group optionally containing a carbonyl group, a carboxyl group, a carboxamide group, an alcohol group, or an ether group, the R^9 group further optionally containing one or more halogen atoms;

with the proviso that when R^6 is $-\text{CH}_3$, and R^7 is $\text{n-C}_{13}\text{H}_{27}$, X^2 is not $-\text{NHC}_2\text{H}_5$.

In a preferred embodiment, R^6 is $\text{C}_1\text{-C}_{10}$ alkyl, cycloalkyl, alkenyl, aryl, arylalkyl, or
15 alkylaryl. In a more preferred embodiment, R^6 is $-\text{CH}_3$.

In another preferred embodiment, R^9 is $-\text{CH}_2\text{C}(\text{O})\text{OR}^{10}$ or $-\text{CH}_2\text{C}(\text{O})\text{NHR}^{10}$, where R^{10} is $\text{C}_1\text{-C}_{10}$ alkyl, cycloalkyl, alkenyl, aryl, arylalkyl, or alkylaryl.

Another embodiment of the invention is compounds of formula III:



III

wherein

$R^{11} = H$, or C_1 - C_{20} alkyl, cycloalkyl, alkenyl, aryl, arylalkyl, or alkylaryl, $=CHR^{13}$, $-C(O)OR^{13}$, $-C(O)R^{13}$, $-CH_2C(O)OR^{13}$, $-CH_2C(O)NHR^{13}$, where R^{13} is H or C_1 - C_{10} alkyl, cycloalkyl, or alkenyl;

5 $R^{12} = C_1$ - C_{20} alkyl, cycloalkyl, alkenyl, aryl, arylalkyl, or alkylaryl;

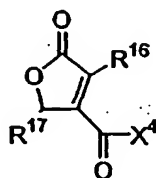
$X^3 = OR^{14}$, where R^{14} is C_1 - C_{20} alkyl, cycloalkyl, alkenyl, aryl, arylalkyl, or alkylaryl, the R^{14} group optionally containing a carbonyl group, a carboxyl group, a carboxamide group, an alcohol group, or an ether group, the R^{14} group further optionally containing one or more halogen atoms.

10 In a preferred embodiment, R^{11} is C_1 - C_{10} alkyl, cycloalkyl, alkenyl, aryl, arylalkyl, or alkylaryl; or $=CH_2$. In a more preferred embodiment, R^{11} is $-CH_3$ or $=CH_2$.

In another preferred embodiment, R^{14} is $-CH_2C(O)OR^{15}$ or $-CH_2C(O)NHR^{15}$, where R^{15} is C_1 - C_{10} alkyl, cycloalkyl, alkenyl, aryl, arylalkyl, or alkylaryl.

Another embodiment of the invention is compounds of formula IV:

15



IV

wherein

20 $R^{16} = H$, or C_1 - C_{20} alkyl, cycloalkyl, alkenyl, aryl, arylalkyl, or alkylaryl, $-C(O)OR^{18}$, $-C(O)R^{18}$, $-CH_2C(O)OR^{18}$, $-CH_2C(O)NHR^{18}$, where R^{18} is H or C_1 - C_{10} alkyl, cycloalkyl, or alkenyl;

$R^{17} = C_1$ - C_{20} alkyl, cycloalkyl, alkenyl, aryl, arylalkyl, or alkylaryl;

$X^4 = OR^{19}$, where R^{19} is C_1 - C_{20} alkyl, cycloalkyl, alkenyl, aryl, arylalkyl, or alkylaryl, the R^{19} group optionally containing a carbonyl group, a carboxyl group, a carboxamide group, an alcohol group, or an ether group, the R^{19} group further optionally containing one or more halogen atoms,

5 with the proviso that when R^{16} is $-CH_3$ and R^{19} is $-CH_3$, then R^{17} is not substituted or unsubstituted phenyl, $-nC_3H_7$, $-nC_5H_{11}$, or $-nC_{13}H_{27}$,

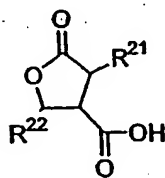
and with the further proviso that when R^{16} is H and R^{19} is $-CH_3$, then R^{17} is not substituted or unsubstituted phenyl or $-CH_3$, and when R^{16} is H and R^{19} is $-CH_2CH_3$, then R^{17} is not $-iC_3H_7$, or substituted or unsubstituted phenyl.

10 In a preferred embodiment, R^{16} is C_1 - C_{10} alkyl, cycloalkyl, alkenyl, aryl, arylalkyl, or alkylaryl. In a more preferred embodiment, R^{16} is $-CH_3$.

In another preferred embodiment, R^{19} is $-CH_2C(O)OR^{20}$ or $-CH_2C(O)NHR^{20}$, where R^{20} is C_1 - C_{10} alkyl, cycloalkyl, alkenyl, aryl, arylalkyl, or alkylaryl.

Another embodiment of the invention is compounds of formula V:

15



V

wherein

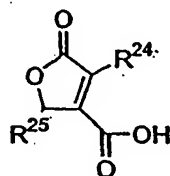
20 $R^{21} = C_2$ - C_{20} alkyl, cycloalkyl, alkenyl, aryl, arylalkyl, or alkylaryl, $=CHR^{23}$, $-C(O)OR^{23}$, $-C(O)R^{23}$, $-CH_2C(O)OR^{23}$, $-CH_2C(O)NHR^{23}$, where R^{23} is H or C_1 - C_{10} alkyl, cycloalkyl, or alkenyl, except when R^{21} is $=CHR^{23}$, R^{23} is not H;

$R^{22} = C_1-C_{20}$ alkyl, cycloalkyl, alkenyl, aryl, arylalkyl, or alkylaryl;

with the proviso that when R^{21} is $-COOH$, then R^{22} is not $-CH_3$, $-nC_5H_{11}$, or $C_{13}H_{27}$; and with the further proviso that when R^{21} is $-CH_2COOH$, then R^{22} is not $-CH_3$, $-CH_2CH_3$, or $-iC_5H_{11}$, and the further proviso that when R^{21} is $=CHCH_3$, then R^{22} is not $n-C_5H_{11}$.

5. In a preferred embodiment, R^{21} is C_2-C_{10} alkyl, cycloalkyl, alkenyl, aryl, arylalkyl, or alkylaryl.

Another embodiment of the invention is compounds of formula VI:



VI

wherein

$R^{24} = C_2-C_{20}$ alkyl, cycloalkyl, alkenyl, aryl, arylalkyl, or alkylaryl, $-C(O)OR^{26}$, $-C(O)R^{26}$,

$-CH_2C(O)OR^{26}$, $-CH_2C(O)NHR^{26}$, where R^{26} is H or C_1-C_{10} alkyl, cycloalkyl, or alkenyl;

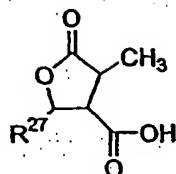
15 $R^{25} = C_1-C_{20}$ alkyl, cycloalkyl, alkenyl, aryl, arylalkyl, or alkylaryl;

with the proviso that when R^{24} is $-COOH$, then R^{25} is not $-CH_3$, $-nC_5H_{11}$, or $C_{13}H_{27}$, and with

the further proviso that when R^{24} is $-CH_2COOH$, then R^{25} is not $-CH_3$, $-CH_2CH_3$, or $-iC_5H_{11}$.

In a preferred embodiment, R^{21} is C_2-C_{10} alkyl, cycloalkyl, alkenyl, aryl, arylalkyl, or
20 alkylaryl.

Another embodiment of the invention is compounds of formula VII:

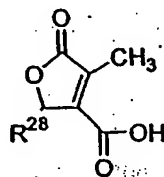


VII

wherein

- 5 $R^{27} = C_3-C_4$ alkyl, C_6-C_{10} alkyl, C_{12} alkyl, C_{14} alkyl, $C_{16}-C_{20}$ alkyl.

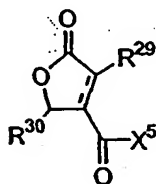
Another embodiment of the invention is compounds of formula VIII:



VIII

wherein R^{28} is C_1-C_{20} alkyl, cycloalkyl, alkenyl, aryl, arylalkyl, or alkylaryl, with the proviso that R^{28} is not $-CH_3$, $-nC_3H_7$, $-nC_{11}H_{23}$, or $-nC_{13}H_{27}$.

- 15 Another embodiment of the invention is pharmaceutical compositions comprising a pharmaceutical diluent or carrier and a compound of formula I, II, III, IV, V, VI, VII, VIII, or IX:



IX

- 20 $R^{29} = H$, or C_1-C_{20} alkyl, cycloalkyl, alkenyl, aryl, arylalkyl, or alkylaryl, $=CHR^{31}$, $-C(O)OR^{31}$, $-C(O)R^{31}$, $-CH_2C(O)OR^{31}$, $-CH_2C(O)NHR^{31}$, where R^{31} is H or C_1-C_{10} alkyl, cycloalkyl, or alkenyl;

$R^{30} = C_1-C_{20}$ alkyl, cycloalkyl, alkenyl, aryl, arylalkyl, or alkylaryl;

$X^5 = -OR^{32}$, or $-NHR^{32}$, where R^{32} is H, C_1-C_{20} alkyl, cycloalkyl, alkenyl, aryl, arylalkyl, or alkylaryl, the R^{32} group optionally containing a carbonyl group, a carboxyl group, a carboxyamide group, an alcohol group, or an ether group, the R^{32} group further optionally
5 containing one or more halogen atoms;

with the proviso that when R^{29} is $=CH_2$, then X^5 is not $-OH$.

In a preferred embodiment, R^{29} is C_1-C_{10} alkyl, cycloalkyl, alkenyl, aryl, arylalkyl, or alkylaryl, or $=CH_2$. In a more preferred embodiment, R^{29} is $-CH_3$ or $=CH_2$.

In another preferred embodiment, R^{32} is $-CH_2C(O)OR^{33}$ or $-CH_2C(O)NHR^{33}$,
10 where R^{33} is C_1-C_{10} alkyl, cycloalkyl, alkenyl, aryl, arylalkyl, or alkylaryl.

The compositions of the present invention can be presented for administration to humans and other animals in unit dosage forms, such as tablets, capsules, pills, powders, granules, sterile parenteral solutions or suspensions, oral solutions or suspensions, oil in water and water in oil emulsions containing suitable quantities of the compound, suppositories and in
15 fluid suspensions or solutions. As used in this specification, the terms "pharmaceutical diluent" and "pharmaceutical carrier," have the same meaning. For oral administration, either solid or fluid unit dosage forms can be prepared. For preparing solid compositions such as tablets, the compound can be mixed with conventional ingredients such as talc, magnesium stearate, dicalcium phosphate, magnesium aluminum silicate, calcium sulfate, starch, lactose, acacia,
20 methylcellulose and functionally similar materials as pharmaceutical diluents or carriers.

Capsules are prepared by mixing the compound with an inert pharmaceutical diluent and filling the mixture into a hard gelatin capsule of appropriate size. Soft gelatin capsules are prepared by

machine encapsulation of a slurry of the compound with an acceptable vegetable oil, light liquid petrolatum or other inert oil.

Fluid unit dosage forms or oral administration such as syrups, elixirs, and suspensions can be prepared. The forms can be dissolved in an aqueous vehicle together with sugar, aromatic flavoring agents and preservatives to form a syrup. Suspensions can be prepared with an aqueous vehicle with the aid of a suspending agent such as acacia, tragacanth, methylcellulose and the like.

For parenteral administration fluid unit dosage forms can be prepared utilizing the compound and a sterile vehicle. In preparing solutions the compound can be dissolved in water for injection and filter sterilized before filling into a suitable vial or ampoule and sealing.

Adjuvants such as a local anesthetic, preservative and buffering agents can be dissolved in the vehicle. The composition can be frozen after filling into a vial and the water removed under vacuum. The lyophilized powder can then be sealed in the vial and reconstituted prior to use.

The clinical therapeutic indications envisioned for the compounds of the invention include: (1) infections due to invasive micro-organisms such as *staphylococci* and *enterococci*; (2) cancers arising in many tissues whose cells over-express fatty acid synthase, and (3) obesity due to the ingestion of excess calories. Dose and duration of therapy will depend on a variety of factors, including (1) the patient's age, body weight, and organ function (e.g., liver and kidney function); (2) the nature and extent of the disease process to be treated, as well as any existing significant co-morbidity and concomitant medications being taken, and (3) drug-related parameters such as the route of administration, the frequency and duration of dosing necessary to effect a cure, and the therapeutic index of the drug. In general, does will be chosen to achieve

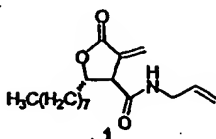
serum levels of 1 ng/ml to 100ng/ml with the goal of attaining effective concentrations at the target site of approximately 1 μ g/ml to 10 μ g/ml.

Examples

5 The invention will be illustrated, but not limited, by the following examples:

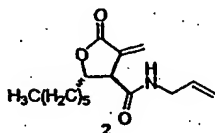
A series of compounds according to the invention were synthesized as described below. Biological activity of certain compounds were profiled as follows: Compounds were tested for: (1) inhibition of purified human FAS, (2) inhibition of fatty acid synthesis activity in whole cells, (3) cytotoxicity against cultured MCF-7 human breast cancer cells, known to possess
10 high levels of FAS and fatty acid synthesis activity, using the crystal violet and XTT assays, and (4) antimicrobial activity. Select compounds with low levels of cytotoxicity were then tested for weight loss in Balb/C mice. In addition, a representative compound from the group which exhibited significant weight loss and low levels of cytotoxicity was tested for its effect on fatty acid oxidation, and carnitine palmitoyltransferase-1 (CPT-1) activity, as well as hypothalamic
15 NPY expression by Northern analysis in Balb/C mice. Certain compounds were also tested for activity against gram positive and/or negative bacteria. Certain compounds were also tested *in vivo* for anti-tumor activity.

Preparation of the compounds

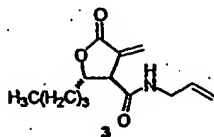


20 (±)- α -Methylene- γ -butyrolactone-5-octyl-4-allyl amide. (1) To a solution of (±)- α -Methylene- γ -butyrolactone-5-octyl-4-carboxylic acid (C75), (40 mg, 0.16 mmol) in CH₃CN (0.9 mL) was added tris (2-oxo-3-oxazolinyl)phosphine oxide¹ (91.7mg, 0.2 mmol), allylamine (12 μ l, 0.2

mmol) and NEt_3 (0.04 mL, 0.3 mmol) and the solution was allowed to stir for 30 min at rt. The mixture was poured into a solution of $\text{NH}_4\text{Cl}_{(\text{sat})}$ /1 N HCl (10 mL, 3:1) and extracted with Et_2O (3 x 15 mL). The combined organics were dried (MgSO_4), filtered, evaporated and chromatographed (35% EtOAc/Hexanes) to give pure 1 (26.2 mg, 54 %); mp. 66-68 °C. ^1H NMR (300 MHz, CDCl_3) δ 0.84 (t, J = 6 Hz, 3 H), 1.23 (m, 11 H), 1.34-1.47 (m, 1 H), 1.60-1.71 (m, 2 H), 3.43-3.46 (m, 1 H), 3.87 (dt, J = 1.4, 5.7 Hz, 2 H), 4.74 (dt, J = 5, 7 Hz, 1 H), 5.12 (d, J = 10.6 Hz, 1 H), 5.16 (d, J = 17.3 Hz, 1 H), 5.72-5.85 (m, 1 H), 5.76 (d, J = 2.6 Hz, 1 H), 6.34 (d, J = 2.6 Hz, 1 H), 6.50 (bs, 1 H). ^{13}C NMR (75 MHz, CDCl_3) δ 14.0, 22.6, 24.9, 29.1, 29.2, 29.4, 31.8, 35.9, 42.3, 52.2, 80.5, 117.0, 124.3, 133.5, 135.4, 168.6, 168.6. IR (NaCl) 2922, 1771, 1756, 1642 1557 cm^{-1} . Anal. Calcd for $\text{C}_{17}\text{H}_{27}\text{NO}_3$: C, 69.5; H, 9.28; Found: C, 69.5; H, 9.09.

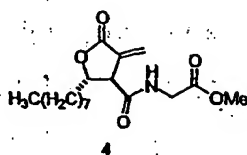


(±)- α -Methylene- γ -butyrolactone-5-hexyl-4-allyl amide (2). From (±)- α -Methylene- γ -butyrolactone-5-hexyl-4-carboxylic acid. (60 mg, 0.27 mmol) and allyl amine (33 μL , 0.29 mmol) following the above procedure was obtained 2 (51.8 mg, 74 %) after flash chromatography (30-40% EtOAc/Hexanes). ^1H NMR (300 MHz, CDCl_3) δ 0.86 (t, J = 6 Hz, 3H), 1.26-1.52 (m, 8 H), 1.63-1.77 (m, 2 H), 3.40-3.43 (m, 1 H), 3.91 (app tt, J = 5.76, 1.44 Hz, 2 H), 4.72-4.78 (m, 1 H), 5.14-5.20 (m, 2 H), 5.75-5.87 (m, 1 H), 5.78 (d, J = 2.4 Hz, 1 H), 5.93 (bt, 1 H), 6.41 (d, J = 2.9 Hz, 1 H); ^{13}C NMR (75 MHz, CDCl_3) δ 13.7, 22.3, 24.7, 28.8, 31.5, 35.9, 42.3, 52.4, 80.3, 116.9, 123.9, 133.5, 135.6, 168.4, 168.5. IR (NaCl) 2923, 1755, 1641, 1557 cm^{-1} . Anal. Calcd for $\text{C}_{15}\text{H}_{23}\text{NO}_3$: C, 67.9; H, 8.74; Found: C, 67.8; H, 8.67.

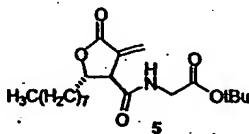


(±)- α -Methylene- γ -butyrolactone-5-butyl-4-allyl amide (3). From (±)- α -Methylene- γ -butyrolactone-5-butyl-4-carboxylic acid. (100 mg, 0.50 mmol) and allyl amine (41 μL , 0.55 mmol) following the above procedure was obtained 3 (68 mg, 57 %) after flash chromatography

- (30-40% EtOAc/Hexanes). ^1H NMR (300 MHz, CDCl_3) δ 0.87 (t, $J = 6$ Hz, 3 H), 1.28-1.50 (m, 4 H), 1.66-1.74 (m, 2 H), 3.41-3.45 (m, 1 H), 3.90 (app tt, $J = 5.7, 1.4$ Hz, 2 H), 4.72-4.78 (m, 1 H), 5.14-5.20 (m, 2 H), 5.74-5.87 (m, 1 H), 5.78 (d, $J = 2.5$ Hz, 1 H), 6.12 (bt, 1 H), 6.39 (d, $J = 2.8$ Hz, 1 H); ^{13}C NMR (75 MHz, CDCl_3) δ 13.6, 22.2, 26.8, 35.5, 42.3, 52.5, 80.3, 117.0, 123.9, 133.5, 135.5, 168.3, 168.5. IR (NaCl) 2958, 1768, 1652, 1548. Anal. Calcd for $\text{C}_{13}\text{H}_{19}\text{NO}_3$: C, 65.8; H, 8.07; Found: C, 65.8; H, 8.07.

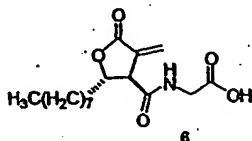


- (±)- α -Methylene- γ -butyrolactone-5-octyl-4-carboxy-methyl glycinate (4). From C75 (39 mg, 0.15 mmol) and methyl glycinate hydrochloride (20 mg, 0.16 mmol) following the above procedure was obtained 4 (28 mg, 56%) after flash chromatography (35% EtOAc/Hexanes); mp. 94.5-95.5 $^{\circ}\text{C}$. ^1H NMR (300 MHz, CDCl_3) δ 0.85 (t, $J = 6.9$ Hz, 3 H), 1.23 (s, 11 H), 1.41-1.49 (m, 1 H), 1.63-1.74 (m, 2 H), 3.46-3.49 (m, 1 H), 3.75 (s, 3 H), 3.97-4.14 (dd, $J = 5.4, 8$ Hz, 2 H), 4.75 (dt, $J = 5.7, 7$ Hz, 1 H), 5.88 (d, $J = 2$ Hz, 1 H), 6.41 (d, $J = 2$ Hz, 1 H), 6.55 (bs, 1 H); ^{13}C NMR (75 MHz, CDCl_3) δ 14.1, 22.6, 24.8, 29.2, 29.2, 29.4, 31.8, 35.8, 41.4, 52.0, 52.6, 80.2, 124.8, 134.9, 168.6, 169.0, 169.9. IR (NaCl) 2915, 1768, 1737, 1644 cm^{-1} ; Anal. Calcd for $\text{C}_{17}\text{H}_{27}\text{NO}_5$: C, 62.7; H, 8.36; Found: C, 62.7; H, 8.27.

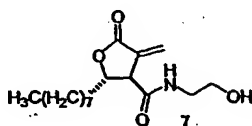


- (±)- α -Methylene- γ -butyrolactone-5-octyl-4-carboxy-*tert*-butyl-glycinate (5). From C75 (100 mg, 0.39 mmol) and *t*-butyl glycinate hydrochloride (66 mg, 0.4 mmol) following the above procedure was obtained 5 (108 mg, 75%) after flash chromatography (35% EtO-30% EtOAc/Hexanes). ^1H NMR (300 MHz, CDCl_3) δ 0.84 (t, $J = 6.8$ Hz, 3 H), 1.25 (s, 12 H), 1.44 (s, 9 H), 1.65-1.73 (m, 2 H), 3.44-3.48 (m, 1 H), 3.92-3.95 (dd, $J = 3.6, 5$ Hz, 2 H), 4.76 (dt, $J = 5.7, 7$ Hz, 1 H), 5.88 (d, $J = 2$ Hz, 1 H), 6.41 (d, $J = 2$ Hz, 1 H), 4.47 (bt, 1 H). ^{13}C NMR (75 MHz,

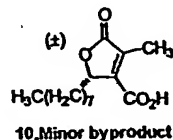
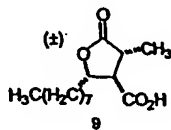
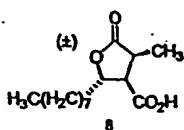
CDCl_3) δ 13.9, 22.5, 24.8, 28.0, 29.1, 29.2, 29.3, 31.7, 35.8, 42.2, 51.9, 80.2, 82.6, 124.6, 135.1, 168.5, 168.6, 168.8. Anal. Calcd for $\text{C}_{20}\text{H}_{33}\text{NO}_6$: C, 65.4, H, 9.05; Found: C, 65.3; H, 9.02.



- 5 (±)- α -Methylene- γ -butyrolactone-5-octyl-4-carboxy-glycinate (6). From 5 (100 mg, 0.27 mmol) in CH_2Cl_2 (2.0 mL) was added TFA (1.3 mL) and the solution was allowed to stir for 3 h at rt. After evaporation of the solvents, column chromatography (50%EtOAc/2% $\text{CH}_3\text{CO}_2\text{H}$ /Hexanes) provided pure 6 (61 mg, 73%). ^1H NMR (300 MHz, MeOD) δ 0.82 (t, J = 7 Hz, 3 H), 1.22 (s, 10 H), 1.28-1.38 (m, 2 H), 1.57-1.69 (m, 2 H), 3.55-3.59 (m, 2 H), 3.78-3.95 (ab-q, J = 17 Hz, 2 H), 4.63 (q_{app}, J = 6.4 Hz, 1 H), 4.88 (bs, 1 H), 5.87 (d, J = 2.6 Hz, 1 H), 6.19 (d, J = 2.6 Hz, 1 H). ^{13}C NMR (75 MHz, MeOD) δ 14.6, 23.8, 26.1, 30.5, 30.5, 30.6, 33.2, 36.6, 42.2, 52.8, 81.7, 124.8, 137.4, 170.8, 172.6, 172.5. IR (NaCl) 2915, 1769, 1731, 1644 cm^{-1} . Anal. Calcd for $\text{C}_{16}\text{H}_{25}\text{NO}_5$: C, 61.7; H, 8.09; Found: C, 61.7; H, 8.05.



- 15 (±)- α -Methylene- γ -butyrolactone-5-octyl-4-carboxylic acid ethanolamide (7). From C75 (30 mg, 0.12 mmol) and ethanolamine (7.8 μl , 0.13 mmol) following the above procedure was obtained 7 (32 mg, 91%) after flash chromatography (50%EtOAc/Hexanes-100% EtOAc/2% $\text{CH}_3\text{CO}_2\text{H}$). ^1H NMR (300 MHz, CDCl_3) δ 0.86 (t, J = 6.9 Hz, 3 H), 1.24 (s, 10 H), 1.35-1.48 (m, 2 H), 1.64-1.75 (m, 2 H), 3.40-3.57 (m, 3 H), 3.74 (t, J = 5 Hz, 2 H), 4.73-4.79 (dt, J = 5.7, 7 Hz, 1 H), 5.82 (d, J = 2 Hz, 1 H), 6.42 (d, J = 2 Hz, 1 H).



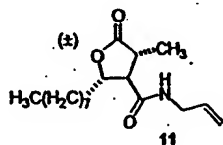
(8,9) To a solution of C75 (100 mg, 0.39 mmol) in EtOAc (3.0 mL) was added Pd (30 mg, 10% on Carbon) and H₂ (50 psi) for 2 h. The mixture was filtered through celite and evaporated to give a mixture of diastereomers (1.8:1 for trans 9:cis 8). Column chromatography (20%EtOAc/2%CH₃CO₂H/ Hexanes) yielded separate trans distereomer with unseparable isomerized byproduct (9:10, 3.8:1, 59.5 mg); and pure cis isomer (8, 32.7 mg,) (92% overall yield).

(±)-α-Methyl-γ-butyrolactone-5-octyl-4-carboxylic acid (Trans diastereomer) (9).

¹H NMR (300 MHz, CDCl₃) δ 0.85 (t, *J* = 7 Hz, 3 H), 1.23 (s, 10 H), 1.31 (d, *J* = 7 Hz, 3 H), 1.41-1.50 (m, 2 H), 1.64-1.69 (m, 2 H), 2.62-2.69 (dd, *J* = 9.6, 11.3 Hz, 1 H), 2.91-3.0 (dq, *J* = 11.3, 7 Hz, 1 H), 4.42-4.49 (td, *J* = 4, 9 Hz, 1 H). ¹³C NMR (75 MHz, CDCl₃) δ 13.9, 14.5, 22.6, 25.2, 29.1, 29.2, 29.3, 31.8, 32.7, 39.9, 53.9, 79.5, 176.0, 176.9. HRMS (ES) *m/z* calculated for C₁₄H₂₄O₄Na⁺ (*M*+Na⁺) 279.1566 observed. 279.1562.

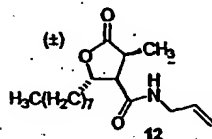
(±)-α-Methyl-γ-butyrolactone-5-octyl-4-carboxylic acid (Cis diastereomer) (8).

¹H NMR (300 MHz, CDCl₃) δ 0.86 (t, *J* = 6.9 Hz, 3 H), 1.25 (bs, 10 H), 1.29 (d, *J* = 7.4 Hz, 3 H), 1.36-1.49 (m, 2 H), 1.63-1.71 (m, 2 H), 3.14 (dd, *J* = 6, 9 Hz, 1 H), 3.02 (dq, *J* = 7, 9 Hz, 1 H), 4.69 (qapp, *J* = 6.3 Hz, 1 H). ¹³C NMR (75 MHz, CDCl₃) δ 11.8, 14.0, 22.6, 25.3, 29.1, 29.2, 29.3, 31.8, 34.7, 37.0, 49.9, 79.5, 175.4, 177.3. HRMS (ES) *m/z* calculated. For C₁₄H₂₄O₄Na⁺ (*M*+Na⁺) 279.1566 observed. 279.1568.

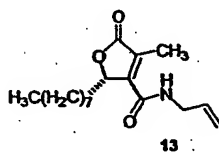


(±)-α-Methyl-γ-butyrolactone-5-octyl-4-carboxylic acid allyl amide (11). From 9 (52 mg, 0.20 mmol) and allyl amine (16 μl, 0.22 mmol) following the above procedure was obtained 11 (30 mg, 51%) after flash chromatography (40%Et₂O/Hexanes- 30% EtOAc/Hexanes). ¹H NMR (300 MHz, CDCl₃) δ 0.86 (t, *J* = 7 Hz, 3 H), 1.23- 1.30 (m, 13 H), 1.38-1.49 (m, 2 H), 1.61-1.69 (m, 2 H), 2.29-2.36 (dd, *J* = 9.3, 11.3 Hz, 1 H), 3.00-3.09 (dq, *J* = 7, 11 Hz, 1 H), 3.92 (tt, *J* = 1.5, 5.7 Hz, 2 H), 4.45-4.52 (m, 1 H), 5.15-5.22 (dd, *J* = 10, 17 Hz, 2 H), 5.76-5.88 (m, 2 H). ¹³C

NMR (75 MHz, CDCl_3) δ 13.9, 14.0, 22.6, 25.4, 29.1, 29.3, 29.3, 31.8, 34.7, 40.5, 42.2, 57.4, 80.4, 116.9, 133.5, 169.3, 177.4. HRMS (ES) m/z calculated for $\text{C}_{17}\text{H}_{29}\text{NO}_3\text{Na}^+$ ($M + \text{Na}^+$) 318.2039; observed 318.2040.



- 5 (±)- α -Methyl- γ -butyrolactone-5-octyl-4-carboxylic acid allyl amide (12). From 8 (32 mg, 0.12 mmol) and allylamine (10 μL , 0.13 mmol) following the above procedure was obtained 12 (20 mg, 53%) after flash chromatography (40% Et_2O /Hexanes-30% EtOAc /Hexanes). ^1H NMR (300 MHz, CDCl_3) δ 0.86 (t, $J = 7$ Hz, 3 H), 1.21-1.25 (m, 13 H), 1.41-1.47 (m, 2 H), 1.58-1.67 (m, 2 H), 2.81-2.91 (m, 2 H), 3.83-3.96 (tt, $J = 1.5, 5$ Hz, 2 H), 4.71-4.77 (m, 1 H), 5.13-5.21 (dd, $J = 10, 17$ Hz, 2 H), 5.75-5.87 (m, 2 H). ^{13}C NMR (75 MHz, CDCl_3) δ 11.5, 14.0, 22.6, 25.4, 29.1, 29.2, 29.4, 31.8, 34.8, 37.4, 42.0, 51.2, 80.3, 116.9, 133.8, 169.1, 177.9. HRMS (ES) m/z calculated for $\text{C}_{17}\text{H}_{29}\text{NO}_3\text{Na}^+$ ($M + \text{Na}^+$) 318.2039; observed 318.2041.



- 15 3-Methyl-5-octyl-5-oxo-2,5-dihydro-furan-3-carboxylic acid allylamide. (13). From 3-Methyl-5-octyl-2-oxo-2,5-dihydro-furan-4-carboxylic acid (46 mg, 0.18 mmol) and allylamine (14 μL , 0.19 mmol) following the above procedure was obtained 13 (30 mg, 55%) after flash chromatography (40% EtOAc /Hexanes. ^1H NMR (300 MHz, CDCl_3) δ 0.85 (t, $J = 6.9$ Hz, 3 H), 1.22 (s, 10 H), 1.46-1.55 (m, 2 H), 1.90-1.95 (m, 2 H), 2.04 (s, 3 H), 4.02 (td, $J = 1.4, 5.7$ Hz, 2 H), 5.13-5.15 (m, 1 H), 5.18-5.25 (dd, $J = 10.6, 17.3$ Hz, 2 H), 5.80-5.92 (ddt, $J = 10.3, 17, 5.7$ Hz, 1 H), 6.07 (t, $J = 1.4$ Hz, 1 H). ^{13}C NMR (75 MHz, CDCl_3) δ 10.3, 14.0, 22.6, 24.8, 29.1, 29.2, 29.3, 31.8, 32.7, 42.0, 81.7, 117.5, 128.8, 133.1, 153.7, 162.1, 173.3. HRMS (ES) m/z calculated for $\text{C}_{17}\text{H}_{27}\text{NO}_3\text{Na}^+$ ($M + \text{Na}^+$) 316.1883 observed 316.1895.

- 25 References: 1. Kunieda, T.; Nagamatsu, T.; Higuchi, T.; Hirobe, M. *Tetrahedron Lett.* 1988, 29, 2203-2206.

BIOLOGICAL AND BIOCHEMICAL METHODS

Purification of FAS from ZR-75-1 Human Breast Cancer Cells.

Human FAS was purified from cultured ZR-75-1 human breast cancer cells obtained from the American Type Culture Collection. The procedure, adapted from Linn *et al.*, 1981, and Kuhajda *et al.*, 1994, utilizes hypotonic lysis, successive polyethyleneglycol (PEG) precipitations, and anion exchange chromatography. ZR-75-1 cells are cultured at 37 °C with 5% CO₂ in RPMI culture medium with 10% fetal bovine serum, penicillin and streptomycin.

Ten T150 flasks of confluent cells are lysed with 1.5 ml lysis buffer (20 mM Tris-HCl, pH 7.5, 1 mM EDTA, 0.1 mM phenylmethanesulfonyl fluoride (PMSF), 0.1% Igepal CA-630) and dounce homogenized on ice for 20 strokes. The lysate is centrifuged in JA-20 rotor (Beckman) at 20,000 rpm for 30 minutes at 4 °C and the supernatant is brought to 42 ml with lysis buffer. A solution of 50% PEG 8000 in lysis buffer is added slowly to the supernatant to a final concentration of 7.5%. After rocking for 60 minutes at 4 °C, the solution is centrifuged in JA-20 rotor (Beckman) at 15,000 rpm for 30 minutes at 4 °C. Solid PEG 8000 is then added to the supernatant to a final concentration of 15%. After the rocking and centrifugation is repeated as above, the pellet is resuspended overnight at 4 °C in 10 ml of Buffer A (20 mM K₂HPO₄, pH 7.4). After 0.45 µM filtration, the protein solution is applied to a Mono Q 5/5 anion exchange column (Pharmacia). The column is washed for 15 minutes with buffer A at 1 ml/minute, and bound material is eluted with a linear 60-ml gradient over 60 minutes to 1 M KCl. FAS (MW~270 kD) typically elutes at 0.25 M KCl in three 0.5 ml fractions identified using 4-15% SDS-PAGE with Coomassie G250 stain (Bio-Rad). FAS protein concentration is determined using the Coomassie Plus Protein Assay Reagent (Pierce) according to manufacturer's specifications

using BSA as a standard. This procedure results in substantially pure preparations of FAS (>95%) as judged by Coomassie-stained gels.

Measurement of FAS Enzymatic Activity and Determination of the IC₅₀ of the Compounds

5 FAS activity is measured by monitoring the malonyl-CoA dependent oxidation of NADPH spectrophotometrically at OD₃₄₀ in 96-well plates (Dils *et al* and Arslanian *et al*, 1975). Each well contains 2 µg purified FAS, 100 mM K₂HPO₄, pH 6.5, 1 mM dithiothreitol (Sigma), and 187.5 µM β-NADPH (Sigma). Stock solutions of inhibitors are prepared in DMSO at 2, 1, and 0.5 mg/ml resulting in final concentrations of 20, 10, and 5 µg/ml when 1 µl of stock is
10 added per well. For each experiment, cerulenin (Sigma) is run as a positive control along with DMSO controls, inhibitors, and blanks (no FAS enzyme) all in duplicate.

The assay is performed on a Molecular Devices SpectraMax Plus Spectrophotometer. The plate containing FAS, buffers, inhibitors, and controls are placed in the spectrophotometer heated to 37°C. Using the kinetic protocol, the wells are blanked on duplicate
15 wells containing 100 µl of 100 mM K₂HPO₄, pH 6.5 and the plate is read at OD₃₄₀ at 10 sec intervals for 5 minutes to measure any malonyl-CoA independent oxidation of NADPH. The plate is removed from the spectrophotometer and malonyl-CoA (67.4 µM, final concentration per well) and acetyl-CoA (61.8 µM, final concentration per well) are added to each well except to the blanks. The plate is read again as above with the kinetic protocol to measure the malonyl-CoA
20 dependent NADPH oxidation. The difference between the Δ OD₃₄₀ for the malonyl-CoA dependent and non-malonyl-CoA dependent NADPH oxidation is the specific FAS activity. Because of the purity of the FAS preparation, non-malonyl-CoA dependent NADPH oxidation is negligible.

The IC_{50} for the compounds against FAS is determined by plotting the ΔOD_{340} for each inhibitor concentration tested, performing linear regression and computing the best-fit line, r^2 values, and 95% confidence intervals. The concentration of compound yielding 50% inhibition of FAS is the IC_{50} . Graphs of ΔOD_{340} versus time are plotted by the SOFTmax PRO software (Molecular Devices) for each compound concentration. Computation of linear regression, best-fit line, r^2 , and 95% confidence intervals are calculated using Prism Version 3.0 (Graph Pad Software).

Crystal Violet Cell Growth Assay

The crystal violet assay measures cell growth but not cytotoxicity. This assay employs crystal violet staining of fixed cells in 96-well plates with subsequent solubilization and measurement of OD_{490} on a spectrophotometer. The OD_{490} corresponds to cell growth per unit time measured. Cells are treated with the compounds of interest or vehicle controls and IC_{50} for each compound is computed.

To measure the cytotoxicity of specific compounds against cancer cells, 5×10^4 MCF-7 human breast cancer cells, obtained from the American Type Culture Collection are plated per well in 24 well plates in DMEM medium with 10% fetal bovine serum, penicillin, and streptomycin. Following overnight culture at $37^\circ C$ and 5% CO_2 , the compounds to be tested, dissolved in DMSO, are added to the wells in 1 μl volume at the following concentrations: 50, 40, 30, 20, and 10 $\mu g/ml$ in triplicate. Additional concentrations are tested if required. 1 μl of DMSO is added to triplicate wells as the vehicle control. C75 is run at 10, and 5 $\mu g/ml$ in triplicate as positive controls.

After 72 hours of incubation, cells are stained with 0.5 ml of Crystal Violet stain (0.5% in 25% methanol) in each well. After 10 minutes, wells are rinsed, air dried, and then solubilized with 0.5 ml 10% sodium dodecylsulfate with shaking for 2 hours. Following transfer of 100 µl from each well to a 96-well plate, plates are read at OD₄₉₀ on a Molecular Devices

5 SpectraMax Plus Spectrophotometer. Average OD₄₉₀ values are computed using SOFTmax Pro Software (Molecular Devices) and IC₅₀ values are determined by linear regression analysis using Prism version 3.02 (Graph Pad Software, San Diego).

XTT Cytotoxicity Assay

10 The XTT assay is a non-radioactive alternative for the [⁵¹Cr] release cytotoxicity assay. XTT is a tetrazolium salt that is reduced to a formazan dye only by metabolically active, viable cells. The reduction of XTT is measured spectrophotometrically as OD₄₉₀ - OD₆₅₀.

To measure the cytotoxicity of specific compounds against cancer cells, 9 x 10³ MCF-7 human breast cancer cells, obtained from the American Type Culture Collection are
15 plated per well in 96 well plates in DMEM medium with 10% fetal bovine serum, insulin, penicillin, and streptomycin. Following overnight culture at 37°C and 5% CO₂, the compounds to be tested, dissolved in DMSO, are added to the wells in 1 µl volume at the following concentrations: 80, 40, 20, 10, 5, 2.5, 1.25, and 0.625 µg/ml in triplicate. Additional concentrations are tested if required. 1 µl of DMSO is added to triplicate wells are the vehicle
20 control. C75 is run at 40, 20, 10, 15, 12.5, 10, and 5 µg/ml in triplicate as positive controls.

After 72 hours of incubation, cells are incubated for 4 hours with the XTT reagent as per manufacturer's instructions (Cell Proliferation Kit II (XTT) Roche). Plates are read at OD₄₉₀ and OD₆₅₀ on a Molecular Devices SpectraMax Plus Spectrophotometer. Three wells

containing the XTT reagent without cells serve as the plate blank. XTT data are reported as OD₄₉₀ - OD₆₅₀. Averages and standard error of the mean are computed using SOFTmax Pro software (Molecular Dynamics).

The IC₅₀ for the compounds is defined as the concentration of drug leading to a 50% reduction in OD₄₉₀ - OD₆₅₀ compared to controls. The OD₄₉₀ - OD₆₅₀ are computed by the SOFTmax PRO software (Molecular Devices) for each compound concentration. IC₅₀ is calculated by linear regression, plotting the FAS activity as percent of control versus drug concentrations. Linear regression, best-fit line, r^2 , and 95% confidence intervals are determined using Prism Version 3.0 (Graph Pad Software).

Measurement of [¹⁴C]acetate Incorporation into Total Lipids and Determination of IC₅₀ of Compounds

This assay measures the incorporation of [¹⁴C]acetate into total lipids and is a measure of fatty acid synthesis pathway activity *in vitro*. It is utilized to measure inhibition of fatty acid synthesis *in vitro*.

MCF-7 human breast cancer cells cultured as above, are plated at 5×10^4 cells per well in 24-well plates. Following overnight incubation, the compounds to be tested, solubilized in DMSO, are added at 5, 10, and 20 µg/ml in triplicate, with lower concentrations tested if necessary. DMSO is added to triplicate wells for a vehicle control. C75 is run at 5 and 10 µg/ml in triplicate as positive controls. After 4 hours of incubation, 0.25 µCi of [¹⁴C]acetate (10 µl volume) is added to each well.

After 2 hours of additional incubation, medium is aspirated from the wells and 800 µl of chloroform:methanol (2:1) and 700 µl of 4 mM MgCl₂ is added to each well. Contents

of each well are transferred to 1.5 ml Eppendorf tubes, and spun at full-speed for 2 minutes in a high-speed Eppendorf Microcentrifuge 5415D. After removal of the aqueous (upper) layer, an additional 700 μ l of chloroform:methanol (2:1) and 500 μ l of 4 mM $MgCl_2$ are added to each tube and then centrifuged for 1 minutes as above. The aqueous layer is removed with a Pasteur
5 pipette and discarded. An additional 400 μ l of chloroform:methanol (2:1) and 200 μ l of 4 mM $MgCl_2$ are added to each tube, then centrifuged and aqueous layer is discarded. The lower (organic) phase is transferred into a scintillation vial and dried at 40 °C under N_2 gas. Once dried, 3 ml of scintillant (APB #NBC5104) is added and vials are counted for ^{14}C . The Beckman Scintillation counter calculates the average cpm values for triplicates.

10 The IC_{50} for the compounds is defined as the concentration of drug leading to a 50% reduction in [^{14}C]acetate incorporation into lipids compared to controls. This is determined by plotting the average cpm for each inhibitor concentration tested, performing linear regression and computing the best-fit line, r^2 values, and 95% confidence intervals. The average cpm values are computed by the Beckman scintillation counter (Model LS6500) for each compound
15 concentration. Computation of linear regression, best-fit line, r^2 , and 95% confidence intervals are calculated using Prism Version 3.0 (Graph Pad Software).

Carnitine Palmitoyltransferase-1 (CPT-1) Assay

CPT-1 catalyzes the ATP dependent transfer of long-chain fatty acids from acyl-
20 CoA to acyl-carnitine that is inhibited by malonyl-CoA. As CPT-1 requires the mitochondrial membrane for activity, enzyme activity is measured in permeabilized cells or mitochondria. This assay uses permeabilized cells to measure the transfer of [methyl- ^{14}C]L-carnitine to the organically soluble acyl-carnitine derivative.

MCF-7 cells are plated in DMEM with 10% fetal bovine serum at 10^6 cells in 24-well plates in triplicate for controls, drugs, and malonyl-CoA. Two hours before commencing the assay, drugs are added at the indicated concentrations made from stock solutions at 10 mg/ml in DMSO, vehicle controls consist of DMSO without drug. Since malonyl-CoA cannot enter intact cells, it is only added in the assay buffer to cells that have not been preincubated with drugs. Following overnight incubation at 37 °C, the medium is removed and replaced with 700 µl of assay buffer consisting of: 50 mM imidazole, 70 mM KCl, 80 mM sucrose, 1 mM EGTA, 2 mM MgCl₂, 1 mM DTT, 1 mM KCN, 1 mM ATP, 0.1% fatty acid free bovine serum albumin, 70 µM palmitoyl-CoA, 0.25 µCi [methyl-¹⁴C]L-carnitine, 40 µg digitonin with drug, DMSO vehicle control, or 20 µM malonyl-CoA. The concentrations of drugs and DMSO in the assay buffer is the same as used in the 2 hr preincubation. After incubation for 6 minutes at 37 °C, the reaction is stopped by the addition of 500 µl of ice-cold 4 M perchloric acid. Cells are then harvested and centrifuged at 13,000 x g for 5 minutes. The pellet is washed with 500 µl ice cold 2mM perchloric acid and centrifuged again. The resulting pellet is resuspended in 800 µl dH₂O and extracted with 150 µl of butanol. The butanol phase is counted by liquid scintillation and represents the acylcarnitine derivative.

Weight Loss Screen for Novel FAS Inhibitors

Balb/C mice (Jackson Labs) are utilized for the initial weight loss screening. Animals are housed in temperature and 12 hour day/night cycle rooms and fed mouse chow and water *ad lib*. Three mice are utilized for each compound tested with vehicle controls in triplicate per experiment. For the experiments, mice are housed separately for each compound tested three mice to a cage. Compounds are diluted in DMSO at 10 mg/ml when given at a dose of

30 mg/kg, and 30 mg/ml when given at a dose of 60 mg/kg, and mice are injected intraperitoneally with 60 mg/kg in approximately 100 μ l of DMSO or with vehicle alone. Mice are observed and weighed daily; average weights and standard errors are computed with Excel (Microsoft). The experiment continues until treated animals reach their pretreatment weights.

- 5 Select compounds are tested in animals housed in metabolic cages.

FIG. 5 shows the results of some *in vivo* testing for weight loss. Dosing of animals are identical to the screening experiments with three animals to a single metabolic cage. Animal weights, water and food consumption, and urine and feces production are measured daily. Three lean Balb/C mice (Harlan) maintained on mouse chow, are treated with compounds at doses indicated on day 0 or with vehicle (DMSO) control of equal volume. Compound 6 was solubilized in 40 μ l DMSO while Compound 8 was solubilized in 60 μ l DMSO. All were injected intraperitoneally. Weights were measured on days indicated. Error bars represent standard error of the mean.

15

Antimicrobial Properties

A broth microdilution assay is used to assess the antimicrobial activity of the compounds. Compounds are tested at twofold serial dilutions, and the concentration that inhibits visible growth (OD₆₀₀ at 10% of control) is defined as the MIC. Microorganisms tested include *Staphylococcus aureus* (ATCC # 29213), *Enterococcus faecalis* (ATCC # 29212), *Pseudomonas aeruginosa* (ATCC # 27853), and *Escherichia coli* (ATCC # 25922). The assay is performed in two growth media, Mueller Hinton Broth and Trypticase Soy Broth.

20

A blood (Tsoy/5% sheep blood) agar plate is inoculated from frozen stocks maintained in T soy broth containing 10% glycerol and incubated overnight at 37° C. Colonies

are suspended in sterile broth so that the turbidity matches the turbidity of a 0.5 McFarland standard. The inoculum is diluted 1:10 in sterile broth (Mueller Hinton or Trypticase soy) and 195 μ l is dispensed per well of a 96-well plate. The compounds to be tested, dissolved in DMSO, are added to the wells in 5 μ l volume at the following concentrations: 25, 12.5, 6.25, 3.125, 1.56 and 0.78 μ g/ml in duplicate. Additional concentrations are tested if required. 5 μ l of DMSO added to duplicate wells are the vehicle control. Serial dilutions of positive control compounds, vancomycin (*E. faecalis* and *S. aureus*) and tobramycin (*E. coli* and *P. aeruginosa*), are included in each run.

After 24 hours of incubation at 37 °C, plates are read at OD₆₀₀ on a Molecular Devices SpectraMax Plus Spectrophotometer. Average OD₆₀₀ values are computed using SOFTmax Pro Software (Molecular Devices) and MIC values are determined by linear regression analysis using Prism version 3.02 (Graph Pad Software, San Diego). The MIC is defined as the concentration of compound required to produce an OD₆₀₀ reading equivalent to 10% of the vehicle control reading.

In Vivo Testing for Anti-Tumor Activity

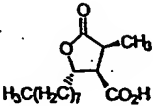
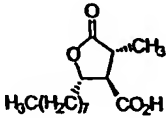
Subcutaneous flank xenografts of the human colon cancer cell line, HCT-116 in nu/nu female mice (Harlan) were used to study the anti-tumor effects of Compound 1 *in vivo*. All animal experiments complied with institutional animal care guidelines. 10⁷ HCT-116 cells (~0.1 ml packed cells) were xenografted from culture in DMEM supplemented with 10% FBS into 20 athymic mice. Treatment began when measurable tumors developed about 3 days after inoculation. Compound 1 (10 mg/kg) was diluted into 40 μ l DMSO and treated intraperitoneally (i.p.) 11 animals received JMM-III-231 10 mg/kg, i.p., at days indicated by

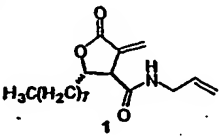
arrows, and 11 received DMSO control. Tumors were measured on days indicated. One Compound 1 treated mouse died on day 10 from repeated i.p. injection. The results are shown in FIG. 4. Error bars represent standard error of the mean.

Subcutaneous flank xenografts of the human colon cancer cell line, HCT-116 in nu/nu female mice (Harlan) were used to study the anti-tumor effects of Compound 7 and Compound 3 *in vivo*. All animal experiments complied with institutional animal care guidelines. 10^7 HCT-116 cells (~0.1 ml packed cells) were xenografted from culture in DMEM supplemented with 10% FBS into 15 athymic mice. Treatment began when measurable tumors developed about 4 days after inoculation. Both Compound 7 and Compound 3 (10 mg/kg) were diluted into 20 μ l DMSO for intraperitoneal (i.p.) injection. 5 animals received drugs i.p. at days indicated by arrows, and 5 received DMSO control. Tumors were measured on days indicated. The results are shown in FIG. 3. Error bars represent standard error of the mean.

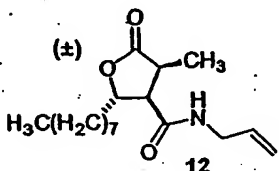
Results of the biological testing

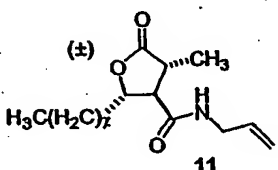
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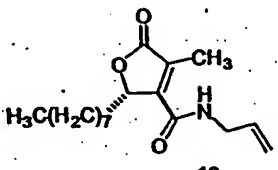
 8	FAS (IC ₅₀)	¹⁴ C (IC ₅₀)	XTT (IC ₅₀)	Cr. Violet (IC ₅₀)
	Neg	31 μ g/ml	>80 μ g/ml	>50 μ g/ml
	CPT I Stim	Weight Loss		
	Not Tested	60mg/kg: 8.6%(day 3); 30 mg/kg: 5.4 % (day2)		
	SA/MH (MIC)	SA/Tsoy(MIC)	PSAE/MH (MIC)	PSAE/Tsoy (MIC)
	Not Tested	Not Tested	Not Tested	Not Tested
	EF/MH (MIC)	EF/Tsoy(MIC)	Ecoli/MH (MIC)	Ecoli/Tsoy (MIC)
 9	FAS (IC ₅₀)	¹⁴ C (IC ₅₀)	XTT (IC ₅₀)	Cr. Violet (IC ₅₀)
	Neg	Neg	>80 μ g/ml	49.0 μ g/ml
	CPT I Stim	Weight Loss		
	Not Tested	Not Tested		
	SA/MH (MIC)	SA/Tsoy(MIC)	PSAE/MH (MIC)	PSAE/Tsoy (MIC)
	43 μ g/ml	60 μ g/ml	Neg	Neg
	EF/MH (MIC)	EF/Tsoy(MIC)	Ecoli/MH (MIC)	Ecoli/Tsoy (MIC)
	109 μ g/ml	94 μ g/ml	Neg	Neg

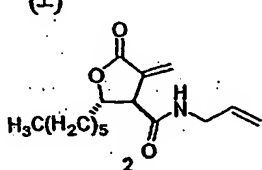
(±) 	FAS (IC ₅₀)	¹⁴ C (IC ₅₀)	XTT (IC ₅₀)	Cr. Violet (IC ₅₀)
	Neg	0.75 ± 0.4 ug/ml	0.81 ± 0.01 ug/ml	0.9 ± 0.5 ug/ml
	CPT I Stim	Weight Loss		
	400% of control/MCF	60 mg/kg: 3 of 3 dead (day 3); 30 mg/kg: 10% (day 2)		
	at 10 ug/ml	20 mg/kg: 11% (day 6); 10 mg/kg: 8.3% (day 7); 5mg/kg: 4.6% (day 1)		
	SA/MH (MIC)	SA/Tsoy (MIC)	PSAE/MH (MIC)	PSAE/Tsoy (MIC)
	9.1 ± 1.9 ug/ml	12.0 ± 0.5 ug/ml	Neg	Neg
	BF/MH (MIC)	BF/Tsoy (MIC)	Ecoli/MH (MIC)	Ecoli/Tsoy (MIC)

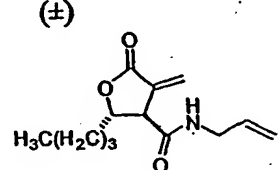
36 ug/ml 25 ug/ml Not Tested Not Tested

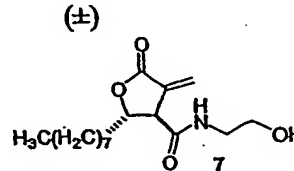
(±) 	FAS (IC ₅₀)	¹⁴ C (IC ₅₀)	XTT (IC ₅₀)	Cr. Violet (IC ₅₀)
	Neg	25.7 ug/ml	59.4 ± 6.4 ug/ml	43.9 ± 4.8 ug/ml
	CPT I Stim	Weight Loss		
	Not Tested	30 mg/kg: 2% (day 1)		
	SA/MH (MIC)	SA/Tsoy (MIC)	PSAE/MH (MIC)	PSAE/Tsoy (MIC)
	107 ug/ml	Neg	Neg	Neg
	BF/MH (MIC)	BF/Tsoy (MIC)	Ecoli/MH (MIC)	Ecoli/Tsoy (MIC)
	91 ug/ml	114 ug/ml	108 ug/ml	Neg

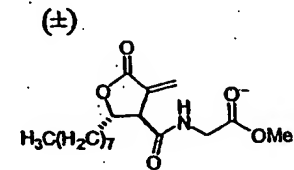
(±) 	FAS (IC ₅₀)	¹⁴ C (IC ₅₀)	XTT (IC ₅₀)	Cr. Violet (IC ₅₀)
	133 ug/ml	Neg	20.8 ± 7.1 ug/ml	30.0 ug/ml
	CPT I Stim	Weight Loss		
	Not Tested	Not Tested		
	SA/MH (MIC)	SA/Tsoy (MIC)	PSAE/MH (MIC)	PSAE/Tsoy (MIC)
	80 ug/ml	193 ug/ml	218 ug/ml	160 ug/ml
	BF/MH (MIC)	BF/Tsoy (MIC)	Ecoli/MH (MIC)	Ecoli/Tsoy (MIC)
	84 ug/ml	Neg	Neg	155 ug/ml

(±) 	FAS (IC ₅₀)	¹⁴ C (IC ₅₀)	XTT (IC ₅₀)	Cr. Violet (IC ₅₀)
	93 ug/ml	Neg (stim)	27.8 ± 3.8 ug/ml	23.7 ug/ml
	CPT I Stim	Weight Loss		
	Not Tested	Not Tested		
	SA/MH (MIC)	SA/Tsoy (MIC)	PSAE/MH (MIC)	PSAE/Tsoy (MIC)
	79 ug/ml	87 ug/ml	280 ug/ml	137 ug/ml
	BF/MH (MIC)	BF/Tsoy (MIC)	Ecoli/MH (MIC)	Ecoli/Tsoy (MIC)
	115 ug/ml	203 ug/ml	Neg	199 ug/ml

(±)  2	FAS (IC ₅₀)	¹⁴ C (IC ₅₀)	XTT (IC ₅₀)	Cr. Violet (IC ₅₀)
	81 ug/ml	3.3 ug/ml	1.6 ± 0.1 ug/ml	0.85 ± 0.08 ug/ml
	CPT I Stim	Weight Loss		
	Not Tested	30 mg/kg: 1 of 3 dead (day1); 10mg/kg: 6.7 % (day4)		
	SA/MH (MIC)	SA/Tsoy(MIC)	PSAE/MH (MIC)	PSAE/Tsoy (MIC)
	49 ug/ml	47 ug/ml	Neg	Neg
	EF/MH (MIC)	EF/Tsoy(MIC)	Ecoli/MH (MIC)	Ecoli/Tsoy (MIC)
	103 ug/ml	38 ug/ml	Neg	Neg

(±)  3	FAS (IC ₅₀)	¹⁴ C (IC ₅₀)	XTT (IC ₅₀)	Cr. Violet (IC ₅₀)
	107 ug/ml	1.8 ± 0.3 ug/ml	2.4 ± 0.2 ug/ml	2.2 ± 0.3 ug/ml
	CPT I Stim	Weight Loss		
	Not Tested	30 mg/kg: 3 of 3 dead (day 2); 10 mg/kg: 4.4% (day 4)		
	SA/MH (MIC)	SA/Tsoy(MIC)	PSAE/MH (MIC)	PSAE/Tsoy (MIC)
	65 ug/ml	96 ug/ml	Neg	Neg
	EF/MH (MIC)	EF/Tsoy(MIC)	Ecoli/MH (MIC)	Ecoli/Tsoy (MIC)
	190 ug/ml	67 ug/ml	Neg	Neg

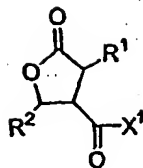
(±)  7	FAS (IC ₅₀)	¹⁴ C (IC ₅₀)	XTT (IC ₅₀)	Cr. Violet (IC ₅₀)
	Neg	2.2 ± 1.3 ug/ml	4.1 ± 2.2 ug/ml	2.2 ± 1.0 ug/ml
	CPT I Stim	Weight Loss		
	Not Tested	30 mg/kg: 5.9%(day2); 10mg/kg: 1.7%(day2)		
	SA/MH (MIC)	SA/Tsoy(MIC)	PSAE/MH (MIC)	PSAE/Tsoy (MIC)
	44 ug/ml	48 ug/ml	Neg	Neg
	EF/MH (MIC)	EF/Tsoy(MIC)	Ecoli/MH (MIC)	Ecoli/Tsoy (MIC)
	Neg	77 ug/ml	Neg	Neg

(±)  4	FAS (IC ₅₀)	¹⁴ C (IC ₅₀)	XTT (IC ₅₀)	Cr. Violet (IC ₅₀)
	Neg	1.1 ± 0.03 ug/ml	2.0 ± 0.5 ug/ml	1.3 ± 0.09 ug/ml
	CPT I Stim	Weight Loss		
	Not Tested	30 mg/kg: 3 of 3 dead (day 1); 10 mg/kg: 3.1% (day2)		
	SA/MH (MIC)	SA/Tsoy(MIC)	PSAE/MH (MIC)	PSAE/Tsoy (MIC)
	4.3 ug/ml	26 ug/ml	Neg	Neg
	EF/MH (MIC)	EF/Tsoy(MIC)	Ecoli/MH (MIC)	Ecoli/Tsoy (MIC)
	80 ug/ml	245 ug/ml	Neg	275 ug/ml

(±) 6	FAS (IC ₅₀)	¹⁴ C (IC ₅₀)	XTT (IC ₅₀)	Cr. Violet (IC ₅₀)
	Neg		>80 ug/ml	>50 ug/ml
	CPT I Stim	Weight Loss		
	Not Tested	60 mg/kg: 9%(day2), 1 death (day3)/ 9.2%(day2), 1 death (day3)		
		30 mg/kg: 6.2%(day1), 1 death (day6)/ 10 mg/kg: 2.6% (day2)		
	SA/MH (MIC)	SA/Tsoy(MIC)	PSAE/MH (MIC)	PSAE/Tsoy (MIC)
	72 ug/ml	52 ug/ml	Neg	Neg
	EF/MH (MIC)	EF/Tsoy(MIC)	Ecoli/MH (MIC)	Ecoli/Tsoy (MIC)
	219 ug/ml	215 ug/ml	Neg	235 ug/ml

We claim:

1. Compounds of formula I:



I

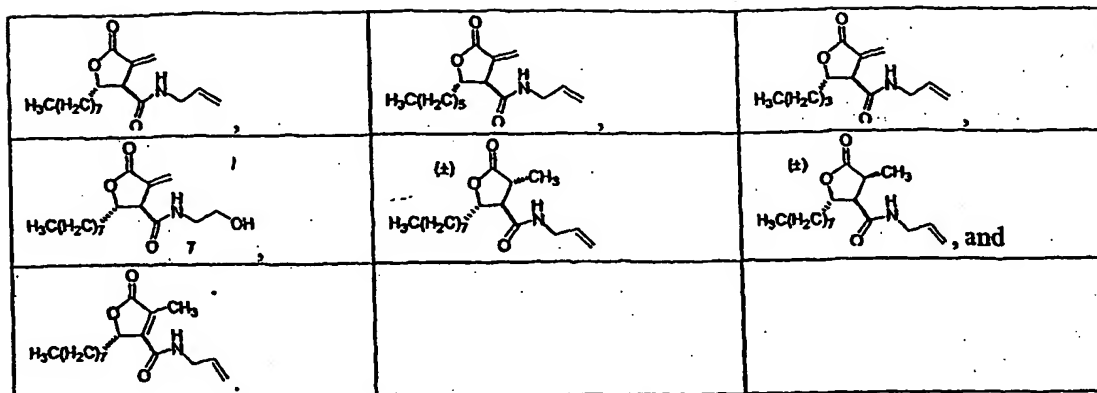
wherein

$R^1 = \text{H}$, or $\text{C}_1\text{-C}_{20}$ alkyl, cycloalkyl, alkenyl, aryl, arylalkyl, or alkylaryl, $=\text{CHR}^3$, $-\text{C}(\text{O})\text{OR}^3$, $-\text{C}(\text{O})\text{R}^3$, $-\text{CH}_2\text{C}(\text{O})\text{OR}^3$, $-\text{CH}_2\text{C}(\text{O})\text{NHR}^3$, where R^3 is H or $\text{C}_1\text{-C}_{10}$ alkyl, cycloalkyl, or alkenyl;

$\text{R}^2 = \text{C}_1\text{-C}_{20}$ alkyl, cycloalkyl, alkenyl, aryl, arylalkyl, or alkylaryl;

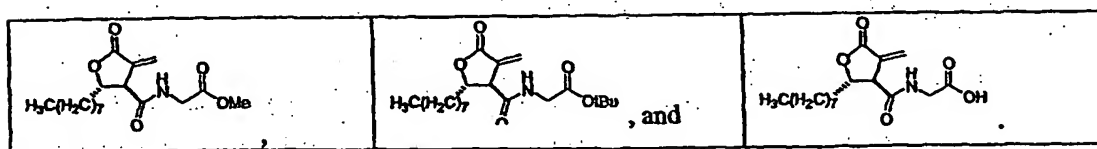
$\text{X}^1 = \text{NHR}^4$, where R^4 is H , $\text{C}_1\text{-C}_{20}$ alkyl, cycloalkyl, alkenyl, aryl, arylalkyl, or alkylaryl, the R^4 group optionally containing a carbonyl group, a carboxyl group, a carboxamide group, an alcohol group, or an ether group, the R^4 group further optionally containing one or more halogen atoms.

2. The compounds of claim 1, wherein R^1 is $\text{C}_1\text{-C}_{10}$ alkyl, cycloalkyl, alkenyl, aryl, arylalkyl, or alkylaryl, or $=\text{CH}_2$.
3. The compounds of claim 2, wherein R^1 is $-\text{CH}_3$ or $=\text{CH}_2$.
4. The compounds of claim 3, wherein the compound is selected from the group consisting of:

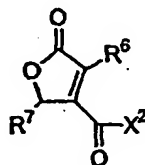


5. The compounds of claim 1, wherein R^4 is $-\text{CH}_2\text{C}(\text{O})\text{OR}^5$ or $-\text{CH}_2\text{C}(\text{O})\text{NHR}^5$, where R^5 is H, $\text{C}_1\text{-C}_{10}$ alkyl, cycloalkyl, alkenyl, aryl, arylalkyl, or alkylaryl.

6. The compounds of claim 5, wherein the compound is selected from the group consisting of:



7. Compounds of formula II:



II

wherein

R^6 = H, or $\text{C}_1\text{-C}_{20}$ alkyl, cycloalkyl, alkenyl, aryl, arylalkyl, or alkylaryl, $-\text{C}(\text{O})\text{OR}^8$, $-\text{C}(\text{O})\text{R}^8$, $-\text{CH}_2\text{C}(\text{O})\text{OR}^8$, $-\text{CH}_2\text{C}(\text{O})\text{NHR}^8$, where R^8 is H or $\text{C}_1\text{-C}_{10}$ alkyl, cycloalkyl, or alkenyl;

R^7 = $\text{C}_1\text{-C}_{20}$ alkyl, cycloalkyl, alkenyl, aryl, arylalkyl, or alkylaryl;

$X^2 = \text{NHR}^9$, where R^9 is H, $C_1\text{-}C_{20}$ alkyl, cycloalkyl, alkenyl, aryl, arylalkyl, or alkylaryl, the R^9 group optionally containing a carbonyl group, a carboxyl group, a carboxyamide group, an alcohol group, or an ether group, the R^9 group further optionally containing one or more halogen atoms;

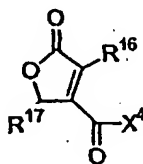
with the proviso that when R^6 is $-\text{CH}_3$, and R^7 is $n\text{-C}_{13}\text{H}_{27}$, X^2 is not $-\text{NHC}_2\text{H}_5$.

8. The compounds of claim 7, wherein R^6 is $C_1\text{-}C_{10}$ alkyl, cycloalkyl, alkenyl, aryl, arylalkyl, or alkylaryl.

9. The compounds of claim 8, wherein R^6 is $-\text{CH}_3$.

10. The compounds of claim 7, wherein R^9 is $-\text{CH}_2\text{C}(\text{O})\text{OR}^{10}$ or $-\text{CH}_2\text{C}(\text{O})\text{NHR}^{10}$, where R^{10} is H, $C_1\text{-}C_{10}$ alkyl, cycloalkyl, alkenyl, aryl, arylalkyl, or alkylaryl.

11. Compounds of formula IV:



IV

wherein

$R^{16} = \text{H}$, or $C_1\text{-}C_{20}$ alkyl, cycloalkyl, alkenyl, aryl, arylalkyl, or alkylaryl, $-\text{C}(\text{O})\text{OR}^{18}$, $-\text{C}(\text{O})\text{R}^{18}$, $-\text{CH}_2\text{C}(\text{O})\text{OR}^{18}$, $-\text{CH}_2\text{C}(\text{O})\text{NHR}^{18}$, where R^{18} is H or $C_1\text{-}C_{10}$ alkyl, cycloalkyl, or alkenyl;

$R^{17} = C_1\text{-}C_{20}$ alkyl, cycloalkyl, alkenyl, aryl, arylalkyl, or alkylaryl;

$X^4 = \text{OR}^{19}$, where R^{19} is $C_1\text{-}C_{20}$ alkyl, cycloalkyl, alkenyl, aryl, arylalkyl, or alkylaryl, the R^{19} group optionally containing a carbonyl group, a carboxyl group, a carboxyamide group, an alcohol group, or an ether group, the R^{19} group further optionally containing one or more halogen atoms;

with the proviso that when R^{16} is $-\text{CH}_3$ and R^{19} is $-\text{CH}_3$, then R^{17} is not substituted or unsubstituted phenyl, $-\text{nC}_3\text{H}_7$, $-\text{nC}_5\text{H}_{11}$, $-\text{nC}_{13}\text{H}_{27}$,

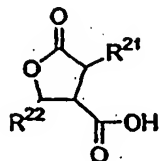
and with the further proviso that when R^{16} is H and R^{19} is $-\text{CH}_3$, then R^{17} is not substituted or unsubstituted phenyl or $-\text{CH}_3$, and when R^{16} is H and R^{19} is $-\text{CH}_2\text{CH}_3$, then R^{17} is not $-\text{iC}_3\text{H}_7$, or substituted or unsubstituted phenyl.

12. The compounds of claim 11, wherein R^{16} is C_1 - C_{10} alkyl, cycloalkyl, alkenyl, aryl, arylalkyl, or alkylaryl.

13. The compounds of claim 12, wherein R^{16} is $-\text{CH}_3$.

14. The compounds of claim 11, wherein R^{19} is $-\text{CH}_2\text{C}(\text{O})\text{OR}^{20}$ or $-\text{CH}_2\text{C}(\text{O})\text{NHR}^{20}$, where R^{20} is C_1 - C_{10} alkyl, cycloalkyl, alkenyl, aryl, arylalkyl, or alkylaryl.

15. Compounds of formula V:



V

wherein

$R^{21} = \text{C}_2$ - C_{20} alkyl, cycloalkyl, alkenyl, aryl, arylalkyl, or alkylaryl, $=\text{CHR}^{23}$, $-\text{C}(\text{O})\text{OR}^{23}$, $-\text{C}(\text{O})\text{R}^{23}$, $-\text{CH}_2\text{C}(\text{O})\text{OR}^{23}$, $-\text{CH}_2\text{C}(\text{O})\text{NHR}^{23}$, where R^{23} is H or C_1 - C_{10} alkyl, cycloalkyl, or alkenyl, except when R^{21} is $=\text{CHR}^{23}$, R^{23} is not H;

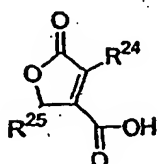
$R^{22} = \text{C}_1$ - C_{20} alkyl, cycloalkyl, alkenyl, aryl, arylalkyl, or alkylaryl;

with the proviso that when R^{21} is $-\text{COOH}$, then R^{22} is not $-\text{CH}_3$, $-\text{nC}_5\text{H}_{11}$, or $\text{C}_{13}\text{H}_{27}$, and with the further proviso that when R^{21} is $-\text{CH}_2\text{COOH}$, then R^{22} is not $-\text{CH}_3$, $-\text{CH}_2\text{CH}_3$, or $-\text{iC}_5\text{H}_{11}$.

16. The compounds of claim 15, wherein R^{21} is C_2 - C_{10} alkyl, cycloalkyl, alkenyl, aryl, arylalkyl, or alkylaryl.

17. The compounds of claim 16, wherein R^{21} is $=CH_2$.

18. Compounds of formula VI:



VI

wherein:

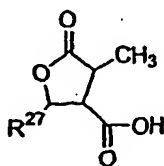
$R^{24} = C_2$ - C_{20} alkyl, cycloalkyl, alkenyl, aryl, arylalkyl, or alkylaryl, $-C(O)OR^{26}$, $-C(O)R^{26}$, $-CH_2C(O)OR^{26}$, $-CH_2C(O)NHR^{26}$, where R^{26} is H or C_1 - C_{10} alkyl, cycloalkyl, or alkenyl;

$R^{25} = C_1$ - C_{20} alkyl, cycloalkyl, alkenyl, aryl, arylalkyl, or alkylaryl;

with the proviso that when R^{24} is $-COOH$, then R^{25} is not $-CH_3$, $-nC_5H_{11}$, or $C_{13}H_{27}$, and with the further proviso that when R^{24} is $-CH_2COOH$, then R^{25} is not $-CH_3$, $-CH_2CH_3$, or $-iC_5H_{11}$.

19. The compounds of claim 18, wherein R^{21} is C_2 - C_{10} alkyl, cycloalkyl, alkenyl, aryl, arylalkyl, or alkylaryl.

20. Compounds of formula VII:

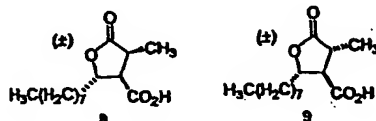


VII

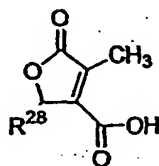
wherein $R^{27} = C_3-C_4$ alkyl, C_6-C_{10} alkyl, C_{12} alkyl, C_{14} alkyl, $C_{16}-C_{20}$ alkyl.

21. The compounds of claim 20, selected from the group consisting of:

and



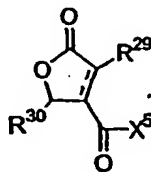
22. A compound of formula VIII:



VIII

wherein R^{28} is C_1-C_{20} alkyl, cycloalkyl, alkenyl, aryl, arylalkyl, or alkylaryl, with the proviso that R^{28} is not $-CH_3$, $-nC_3H_7$, $-nC_{11}H_{23}$, or $-nC_{13}H_{27}$.

23. A pharmaceutical composition comprising a pharmaceutical diluent and a compound of formula IX:



IX

$R^{29} = H$, or C_1-C_{20} alkyl, cycloalkyl, alkenyl, aryl, arylalkyl, or alkylaryl, $=CHR^{31}$, $-C(O)OR^{31}$, $-C(O)R^{31}$, $-CH_2C(O)OR^{31}$, $-CH_2C(O)NHR^{31}$, where R^{31} is H or C_1-C_{10} alkyl, cycloalkyl, or alkenyl;

$R^{30} = C_1-C_{20}$ alkyl, cycloalkyl, alkenyl, aryl, arylalkyl, or alkylaryl;

$X^5 = -OR^{32}$, or $-NHR^{32}$, where R^{32} is H, C_1 - C_{20} alkyl, cycloalkyl, alkenyl, aryl, arylalkyl, or alkylaryl, the R^{32} group optionally containing a carbonyl group, a carboxyl group, a carboxamide group, an alcohol group, or an ether group, the R^{32} group further optionally containing one or more halogen atoms;

with the proviso that when R^{29} is $=CH_2$, then X^5 is not OH.

24. The pharmaceutical compositions of claim 23, wherein R^{29} is C_1 - C_{10} alkyl, cycloalkyl, alkenyl, aryl, arylalkyl, or alkylaryl, or $=CH_2$.

25. The pharmaceutical compositions of claim 24, wherein R^{29} is $-CH_3$ or $=CH_2$.

26. The pharmaceutical compositions of claim 23, wherein R^{32} is $-CH_2C(O)OR^{33}$ or $-CH_2C(O)NHR^{33}$, where R^{33} is C_1 - C_{10} alkyl, cycloalkyl, alkenyl, aryl, arylalkyl, or alkylaryl.

27. The pharmaceutical compositions of claim 23, where R^{29} is $-C_6H_{13}$ or $-C_8H_{17}$.

28. The pharmaceutical compositions of claim 23, wherein the compound is selected from the group consisting of:

29. A pharmaceutical composition comprising a pharmaceutical diluent and a compound according to claim 1.

30. A pharmaceutical composition comprising a pharmaceutical diluent and a compound according to claim 7.

31. A pharmaceutical composition comprising a pharmaceutical diluent and a compound according to claim 11.

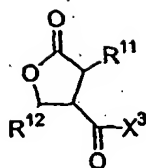
32. A pharmaceutical composition comprising a pharmaceutical diluent and a compound according to claim 15.

33. A pharmaceutical composition comprising a pharmaceutical diluent and a compound according to claim 18.

34. A pharmaceutical composition comprising a pharmaceutical diluent and a compound according to claim 20.

35. A pharmaceutical composition comprising a pharmaceutical diluent and a compound according to claim 22.

36. A pharmaceutical composition comprising a pharmaceutical diluent and a compound according to Formula III:.



III

wherein

R^{11} = H, or C_1 - C_{20} alkyl, cycloalkyl, alkenyl, aryl, arylalkyl, or alkylaryl, $=CH-R^{13}$, $-C(O)OR^{13}$, $-C(O)R^{13}$, $-CH_2C(O)OR^{13}$, $-CH_2C(O)NHR^{13}$, where R^{13} is H or C_1 - C_{10} alkyl, cycloalkyl, or alkenyl;

R^{12} = C_1 - C_{20} alkyl, cycloalkyl, alkenyl, aryl, arylalkyl, or alkylaryl;

$X^3 = OR^{14}$, where R^{14} is C_1 - C_{20} alkyl, cycloalkyl, alkenyl, aryl, arylalkyl, or alkylaryl, the R^{14} group optionally containing a carbonyl group, a carboxyl group, a carboxyamide group, an alcohol group, or an ether group, the R^{14} group further optionally containing one or more halogen atoms.

37. The pharmaceutical formulation of claim 36, wherein R^{11} is C_1 - C_{10} alkyl, cycloalkyl, alkenyl, aryl, arylalkyl, or alkylaryl, or $=CH_2$.

38. The pharmaceutical formulation of claim 37, wherein R^{11} is $-CH_3$ or $=CH_2$.

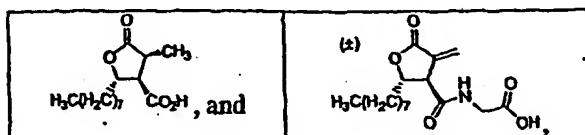
39. The pharmaceutical formulation of claim 36, wherein R^{14} is $-CH_2C(O)OR^{15}$ or $-CH_2C(O)NHR^{15}$, where R^{15} is C_1 - C_{10} alkyl, cycloalkyl, alkenyl, aryl, arylalkyl, or alkylaryl.

40. A method of inducing weight loss in an animal or human subject comprising administering an effective amount of a pharmaceutical composition according to claim 23 to said subject.

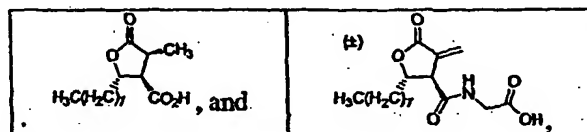
41. The method of claim 40, wherein the subject is a human.

42. The method of claim 40, wherein the subject is an animal.

43. The method of claim 41, wherein the pharmaceutical composition comprises a compound selected from the group consisting of:



44. The method of claim 42, wherein the pharmaceutical composition comprises a compound selected from the group consisting of:

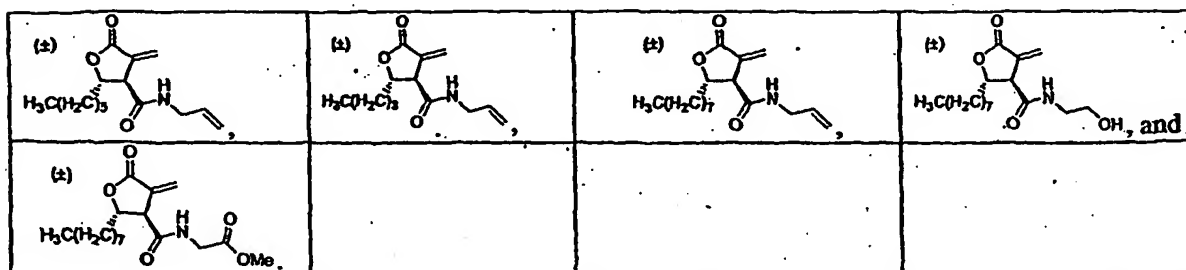


45. A method of inhibiting growth of cancer cells in an animal or human subject, comprising administering an effective amount of a pharmaceutical composition according to claim 23 to said subject.

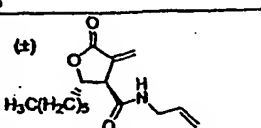
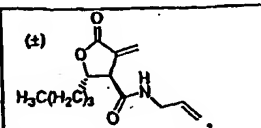
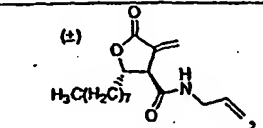
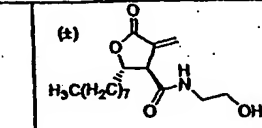
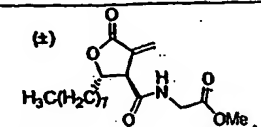
46. The method of claim 45, wherein the subject is a human.--

47. The method of claim 45, wherein the subject is an animal.--

48. The method of claim 46, wherein the pharmaceutical composition comprises a compound selected from the group consisting of:



49. The method of claim 47, wherein the pharmaceutical composition comprises a compound selected from the group consisting of:

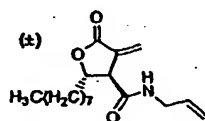
(±) 	(±) 	(±) 	(±) 
(±) 			

50. A method of stimulating the activity of CPT-1 in an animal or human subject comprising administering an effective amount of a pharmaceutical composition according to claim 23 to said subject.

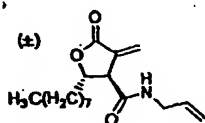
51. The method of claim 50, wherein the subject is a human.

52. The method of claim 50, wherein the subject is an animal.

53. The method of claim 51, wherein the compound is:



54. The method of claim 52, wherein the compound is:



55. A method of inhibiting the activity of neuropeptide-Y in an animal or human subject comprising administering an effective amount of a pharmaceutical composition according to claim 23 to said subject.

56. The method of claim 55, wherein the subject is a human.

57. The method of claim 55, wherein the subject is an animal.

58. A method of inhibiting fatty acid synthase activity in an animal or human subject comprising administering an effective amount of a pharmaceutical composition according to claim 23 to said subject.

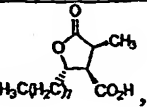
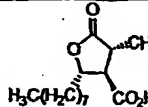
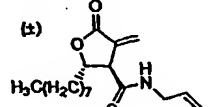
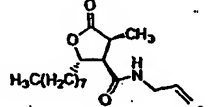
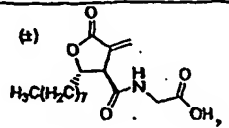
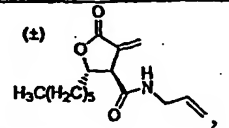
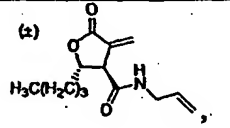
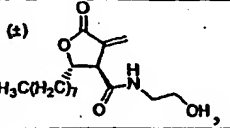
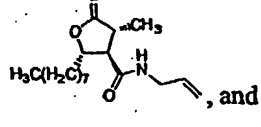
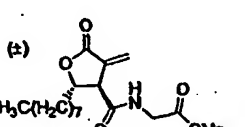
59. The method of claim 58, wherein the subject is a human.

60. The method of claim 58, wherein the subject is an animal.

61. The method of claim 59, wherein the compound is selected from the group consisting of:

		(±)	
(±)	(±)	(±)	(±)
	(±)		

62. The method of claim 60, wherein the compound is selected from the group consisting of:

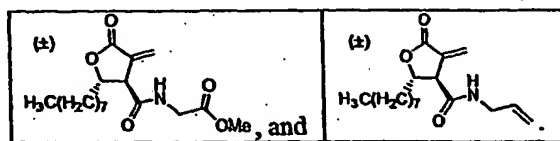
			
			
			

63. A method of inhibiting growth of invasive microbial cells in an animal or human subject comprising the administration of an effective amount of a pharmaceutical composition according to claim 23 to said subject.

64. The method of claim 63, wherein the subject is a human.

65. The method of claim 63, wherein the subject is an animal.

66. The method of claim 64, wherein the compound is selected from the group consisting of:



67. The method of claim 65, wherein the compound is selected from the group consisting of:

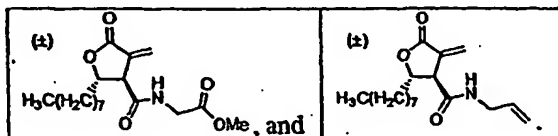


FIG. 1

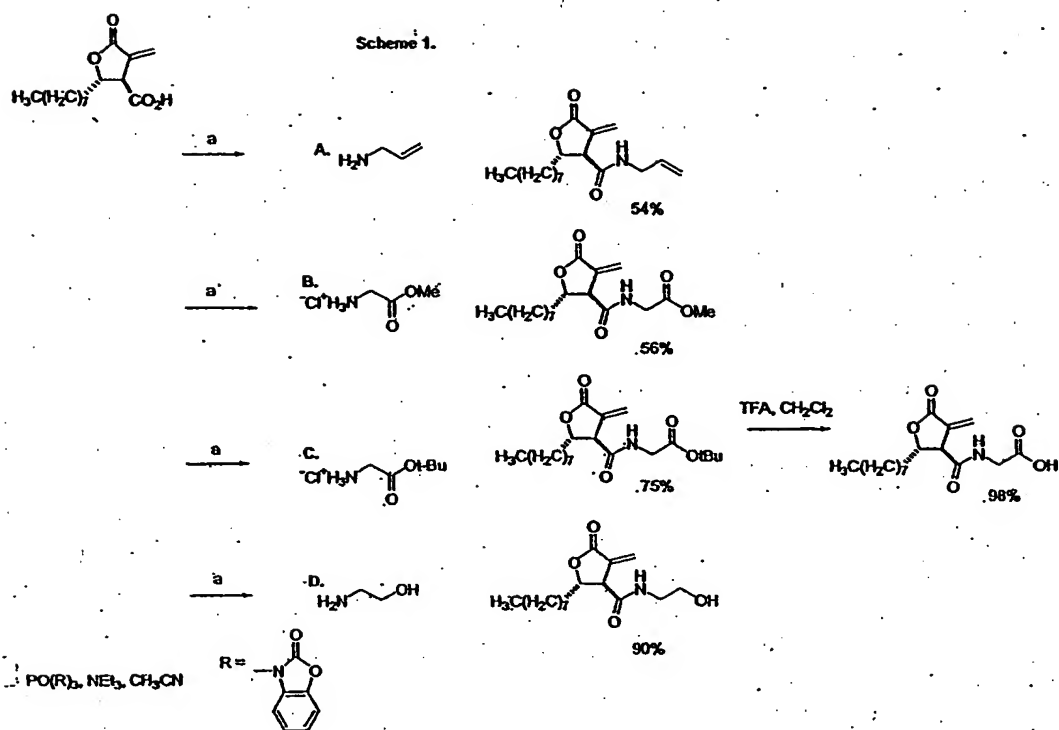


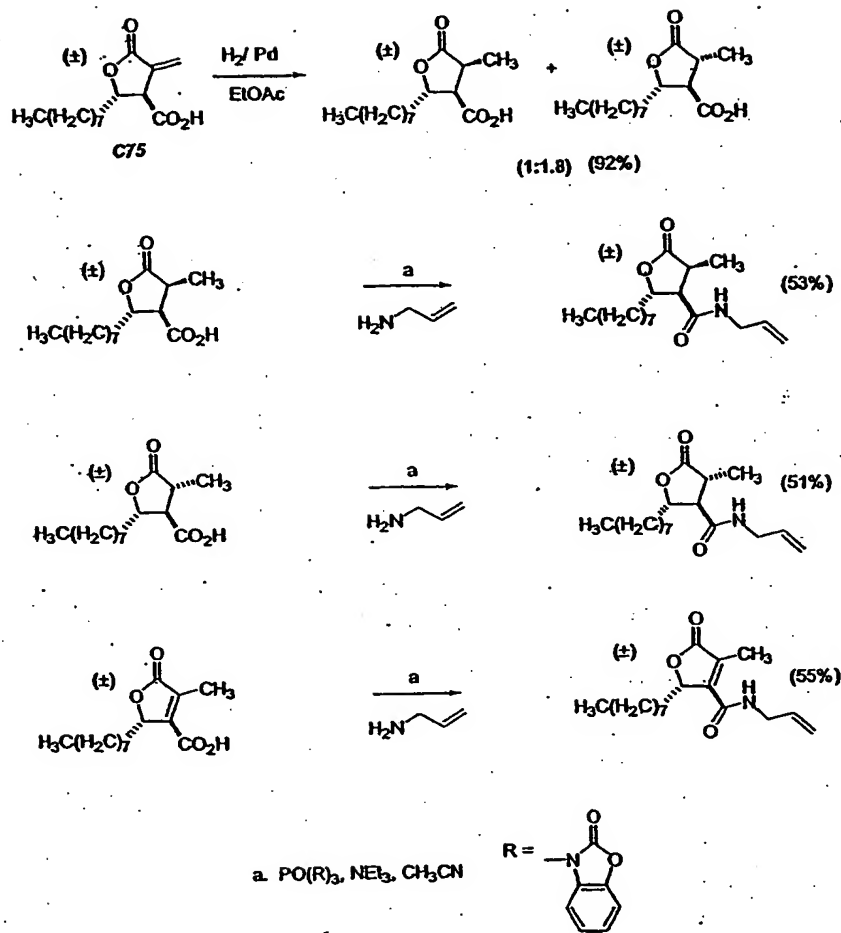
FIG. 2**Scheme 2.**

FIG. 3

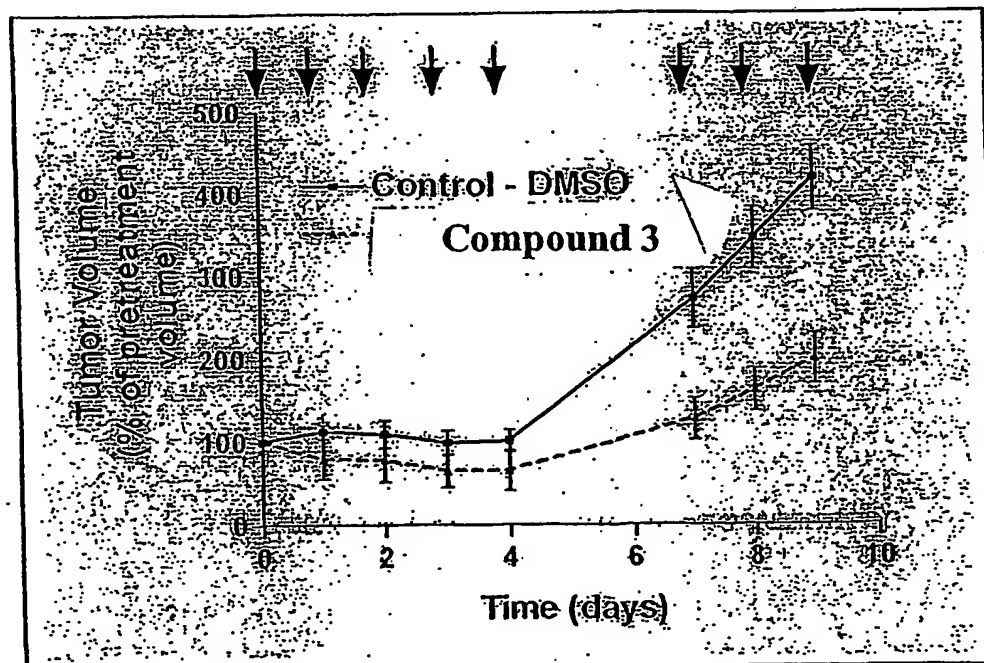
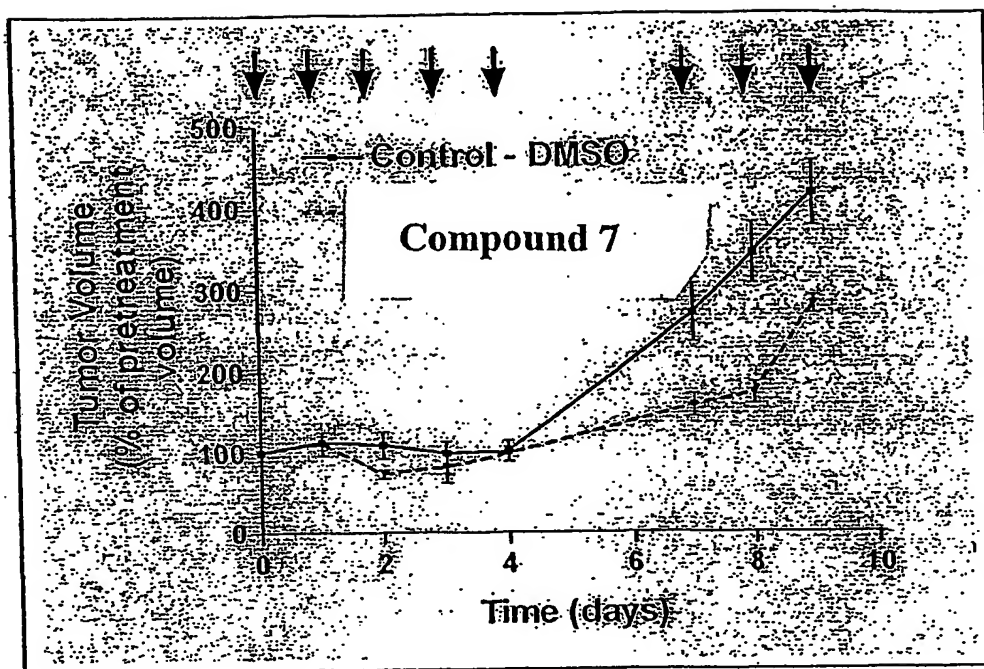


FIG. 4

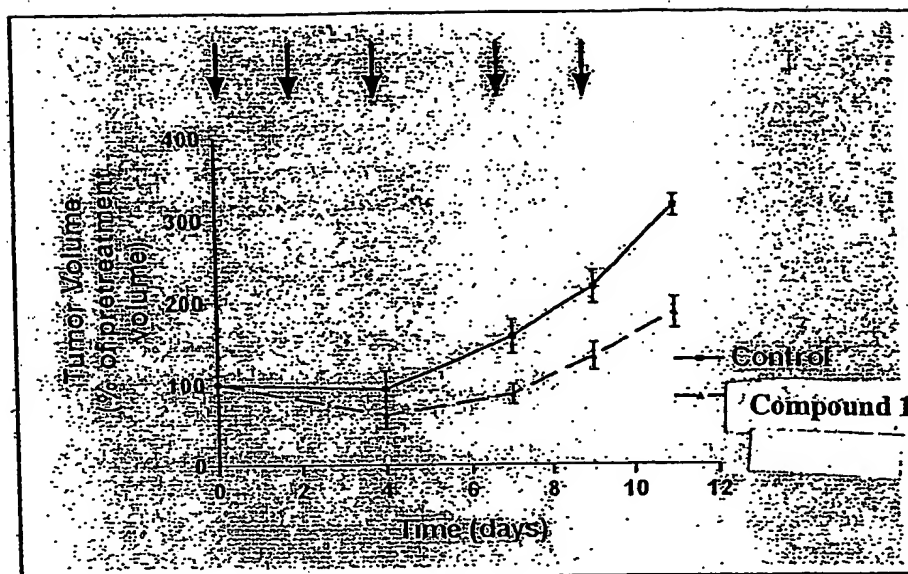
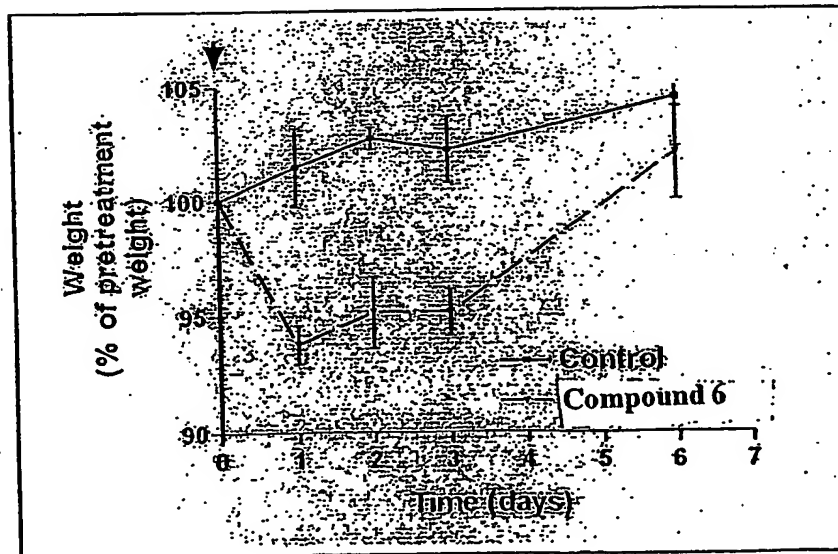
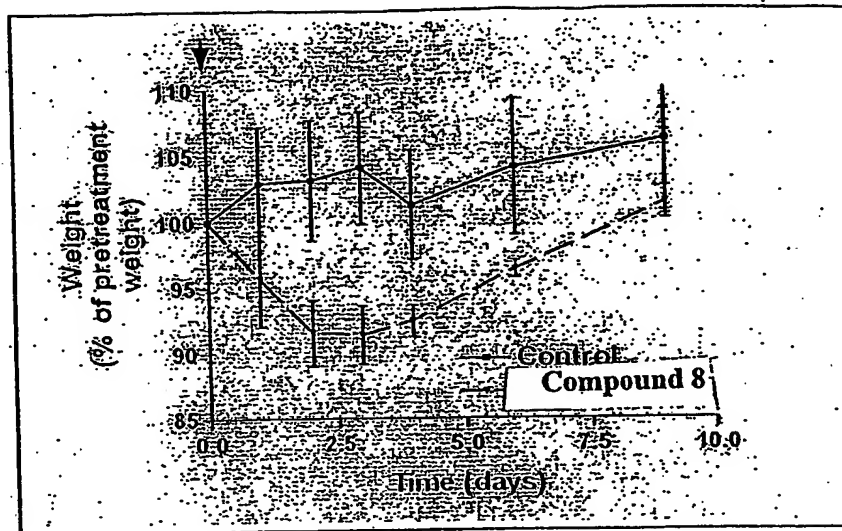


FIG. 5



INTERNATIONAL SEARCH REPORT

International application No.

PCT/US03/20960

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : A61K 31/34; C07D 307/56

US CL : 514/471, 473; 549/321, 322

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
U.S. : 514/471, 473; 549/321, 322

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 3,472,878 A (BRUDERLEIN et al) 14 October 1969 (14.10.1969), column 1 line 49-62.	1-67
A	US 3,496,187 A (BUDERLEIN et al) 17 february 1970 (17.02.1970), column 1 lines 23-48.	1-67

☐ Further documents are listed in the continuation of Box C.

☐ See patent family annex.

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Date of the actual completion of the international search

28 October 2003 (28.10.2003)

Date of mailing of the international search report

18 MAY 2004

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Facsimile No. (703)305-3230

Authorized Officer

Amelia A. Owens

Telephone No. 703-308-1235

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